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C219-E007A

Shimadzu Food Safety Management Data Book

Analysis of Residual Pesticides / Veterinary Medicines / Food Additives

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1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (1) - GC

■Explanation

Gas chromatography (GC) is often employed for analyzing residues of regulated pesticides and, in particular, capillary columns are being used with increased frequency due to their high separation efficiency. This section shows examples of the separation of 124 pesticides, including pesticides with regulated residue levels using four types of capillary columns. The FTD and ECD detectors were used. The pesticides (organochlorinated, organophosphorus and nitrogen-containing) were dissolved in acetone, the mixture concentrations were adjusted to 0.5 to 2.0 mg/L and 2 µL samples were injected into the GC. The analysis results from the two detectors were combined and the relative retention times with EPN as reference were tabulated (the retention time of EPN was assumed to be 30 minutes). It is difficult to simultaneously separate all 124 pesticides with one type of column. However, this relative retention time data can be utilized for screening pesticides before conducting individual analysis.

■DB-1 Separation

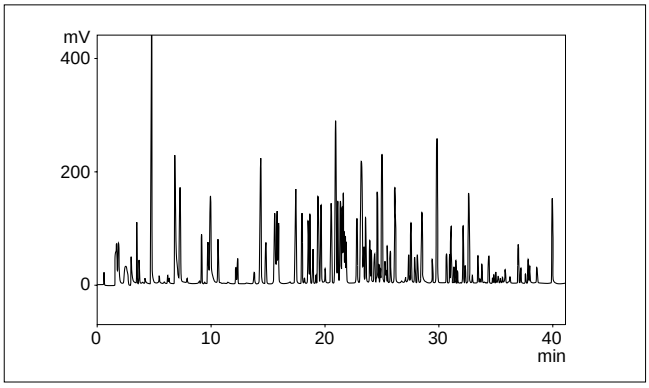


Fig. 1.1.1 Chromatogram for 124 Pesticides Using DB-1
(Full Chromatogram, FTD)

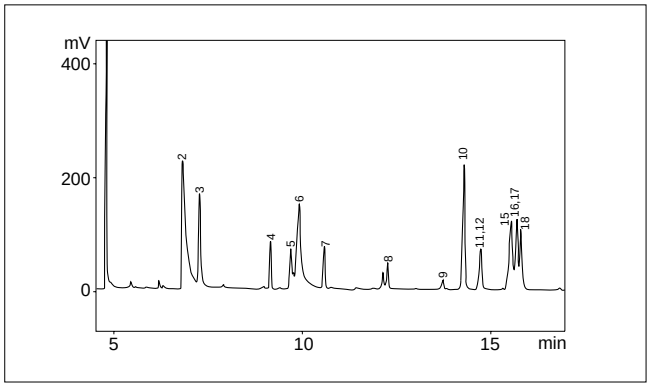


Fig. 1.1.2 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, FTD)

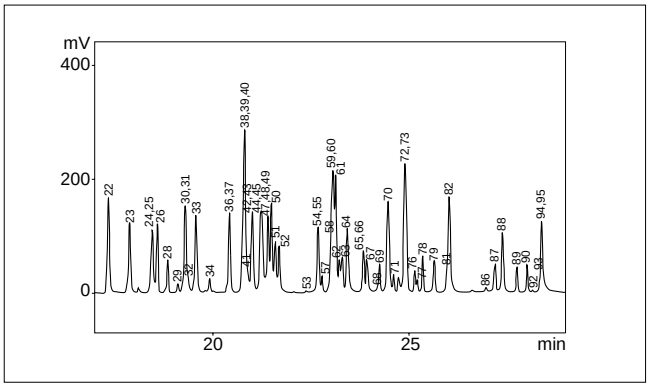


Fig. 1.1.3 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, FTD)

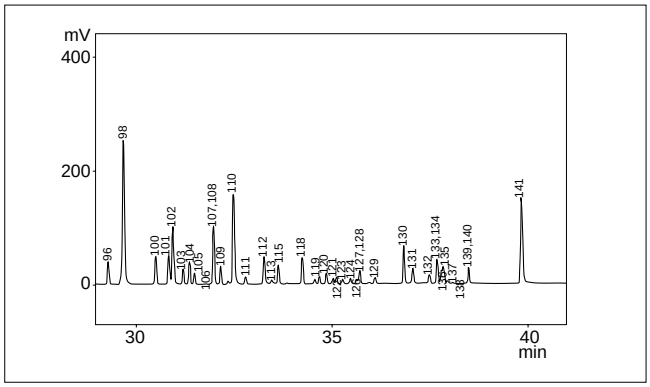


Fig. 1.1.4 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, FTD)

Table 1.1.1 Analytical Conditions

Instrument	: GC-17AAFwFtE ver.3, AOC-20i	Detector	: FTD-17, ECD-17
Column	: DB-1 30m × 0.25mm I.D. df=0.25µm	Detector temperature	: 300°C
Column temperature	: 50°C(1min)-20°C/min-120°C -5°C/min-300°C(10min)	Injection inlet temperature Injection method	: 280°C : High pressure splitless (300kPa 1min)
Carrier Gas	: He, 150kPa	Injection volume	: 2µL

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (2) - GC

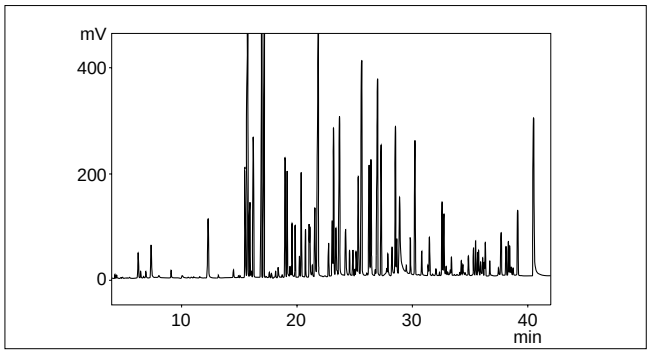


Fig. 1.1.5 Chromatogram for 124 Pesticides Using DB-1
(Full Chromatogram, ECD)

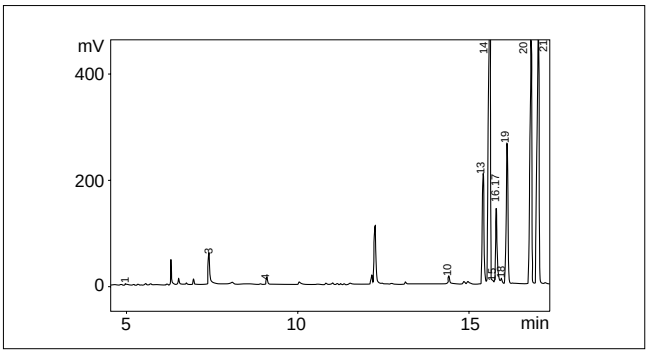


Fig. 1.1.6 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, ECD)

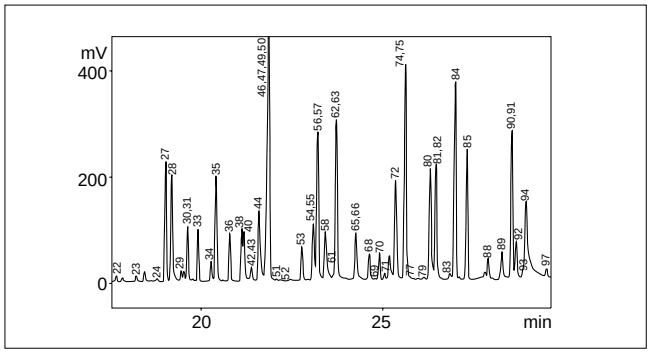


Fig. 1.1.7 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, ECD)

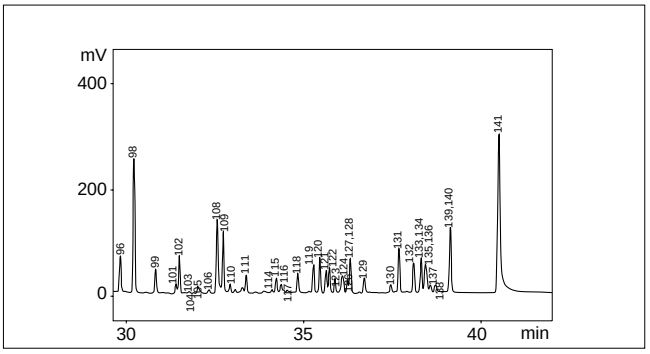


Fig. 1.1.8 Chromatogram for 124 Pesticides Using DB-1
(Enlarged Section, ECD)

Table 1.1.2 Relative Retention Times for 124 Pesticides Using DB-1 (with EPN assumed to be 30 minutes)

Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time
1	DCIP	5.053	37	methiocarb	20.677	73	myclobutanil	25.172	109	cyhalothrin-2	32.506
2	methamidophos	6.973	38	dichlofluanid	20.995	74	dieldrin	25.427	110	pyraclofos	32.788
3	dichlorvos	7.337	39	pirimifos-methyl	21.060	75	DDE(p,p')	25.427	111	acrinathrin	33.167
4	EPTC	9.179	40	dimethylvinphos(E)	21.060	76	difenzoquat methyl sulfate	25.457	112	bitertanol-1	33.616
5	propamocarb	9.827	41	esprocarb	21.136	77	pretilachlor	25.527	113	bitertanol-2	33.797
6	acephate	10.096	42	thiobencarb	21.267	78	flusilazole	25.665	114	permethrin-1	33.878
7	butylate	10.740	43	malathion	21.267	79	cyproconazole	25.947	115	pyridaben	33.987
8	isoprocarb	12.440	44	dimethylvinphos(Z)	21.469	80	endrin	26.103	116	permethrin-2	34.160
9	fenobucarb	13.916	45	fenthion	21.502	81	chlorphenapyr	26.285	117	inabenfide	34.300
10	ethoprophos	14.472	46	aldrin	21.663	82	fensulfothion	26.304	118	cafenstrole	34.617
11	chlorpropham	14.938	47	parathion	21.709	83	chlorobenzilate	26.647	119	cyfluthrin-1	35.042
12	bendiocarb	14.938	48	diethofencarb	21.709	84	DDD(p,p')	26.778	120	cyfluthrin-2	35.232
13	dimethipin	15.443	49	metolachlor	21.709	85	DDT(o,p')	27.104	121	cyfluthrin-3	35.400
14	BHC(α)	15.640	50	chlorpyrifos	21.730	86	mepronil	27.264	122	cyfluthrin-4	35.488
15	cadusafos	15.730	51	fosthiazate-1	21.882	87	lenacil	27.473	123	cypermethrin-1	35.649
16	trifluralin	15.864	52	fosthiazate-2	21.954	88	edifenphos	27.682	124	cypermethrin-2	35.840
17	dimethoate	15.896	53	captan	22.632	89	propiconazole-1	28.058	125	halfenprox	35.883
18	thiometon	16.021	54	pendimethalin	22.952	90	propiconazole-2	28.320	126	cypermethrin-3	36.009
19	BHC(β)	16.159	55	chlorfenvinphos(α)	22.952	91	DDT(p,p')	28.327	127	cypermethrin-4	36.086
20	BHC(γ)	16.862	56	heptachlor epoxide	23.070	92	captafol	28.439	128	flucythrinate-1	36.086
21	BHC(σ)	17.060	57	pyrifenox-1	23.070	93	thenylchlor	28.698	129	flucythrinate-2	36.481
22	terbufos	17.573	58	chlorfenvinphos(β)	23.268	94	acetamiprid	28.736	130	pyrimidifen	37.225
23	diazinon	18.122	59	phenthoate	23.296	95	tebuconazole	28.736	131	fenvalerate-1	37.451
24	etrimfos	18.695	60	quinalphos	23.296	96	pyributicarb	29.648	132	fenvalerate-2	37.869
25	ethiofencarb	18.700	61	isofenphos	23.397	97	dicofol	29.261	133	fluvalinate-1	38.083
26	pirimicarb	18.831	62	triadimenol	23.518	98	EPN	30.000	134	difenoconazole-1	38.083
27	tefluthrin	18.905	63	chinomethionat	23.549	99	bifenthrin	30.634	135	fluvalinate-2	38.193
28	metribuzin	19.075	64	vamidothion	23.698	100	tebufenpyrad	30.859	136	difenoconazole-2	38.227
29	bentazone	19.330	65	pyrifenox-2	24.094	101	furametpyr	31.192	137	pyrazoxyfen	38.329
30	parathion-methyl	19.514	66	paclobutrazol	24.109	102	phosalone	31.258	138	deltamethrin-1	38.475
31	dimethenamid	19.514	67	tricyclazole	24.218	103	mefenacet	31.521	139	deltamethrin-2	38.895
32	carbaryl	19.587	68	trichlamide	24.471	104	pyriproxyfen	31.727	140	tralomethrin	38.895
33	tolclophos-methyl	19.801	69	fludioxonil	24.525	105	cyhalofop-butyl	31.850	141	imibenconazole	40.263
34	alachrol	20.161	70	butamifos	24.754	106	cyhalothrin-1	32.116			
35	heptachlor	20.290	71	flutolanil	24.869	107	amitraz	32.320			
36	fenitrothion	20.671	72	prothiofos	25.172	108	fenarimol	32.362			

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (3) - GC

■DB-5 Separation

Table 1.1.3 Analytical Conditions

Instrument	: GC-17AAFwFtE ver.3, AOC-20i	Detector	: FTD-17, ECD-17
Column	: DB-5(30 m × 0.25 mm I.D. df=0.25μm)	Detector temperature	: 300°C
Column temperature	: 50°C(1min)-20°C/min-120°C	Injection inlet temperature	: 280°C
	-5°C/min-300°C(10min)	Injection method	: High Pressure Splitless(300kPa, 1min)
Carrier Gas	: He, 150kPa	Injection volume	: 2μL

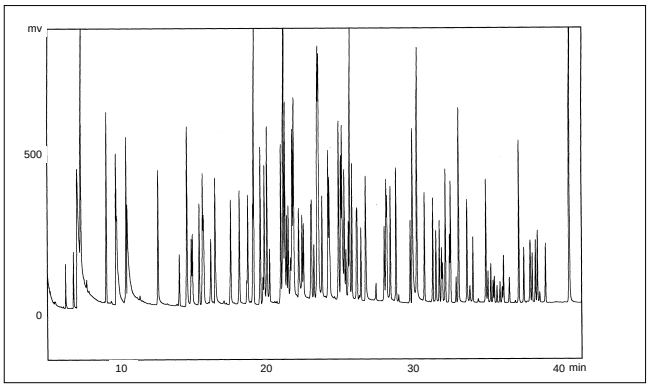


Fig. 1.1.9 Chromatogram for 124 Pesticides Using DB-5
(Full Chromatogram, FTD)

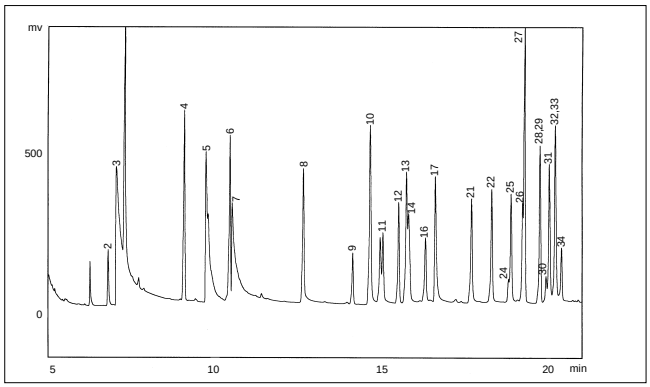


Fig. 1.1.10 Chromatogram for 124 Pesticides Using DB-5
(Enlarged Section, FTD)

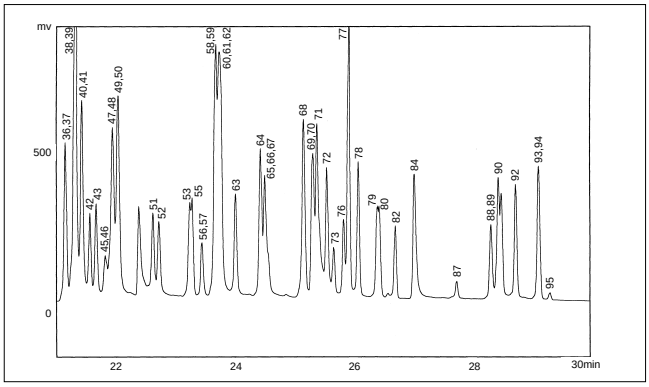


Fig. 1.1.11 Chromatogram for 124 Pesticides Using DB-5
(Enlarged Section, FTD)

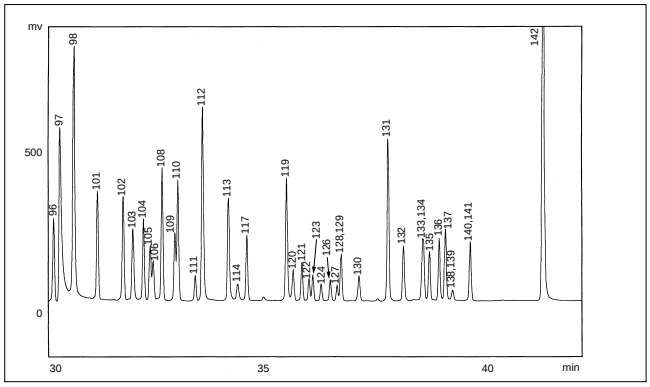


Fig. 1.1.12 Chromatogram for 124 Pesticides Using DB-5
(Enlarged Section, FTD)

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (4) - GC

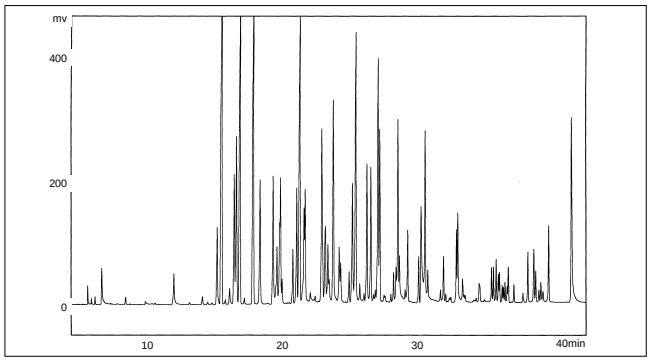


Fig. 1.1.13 Chromatogram for 124 Pesticides Using DB-5 (Full Chromatogram, ECD)

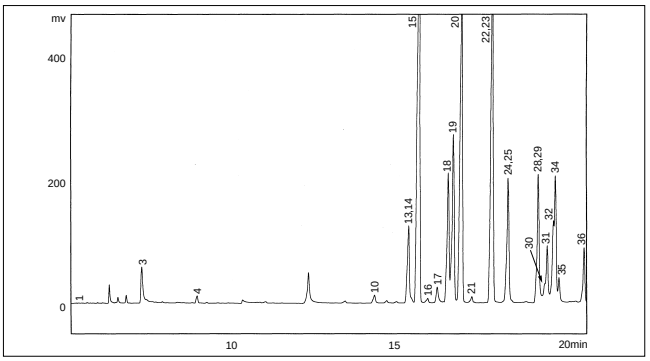


Fig. 1.1.14 Chromatogram for 124 Pesticides Using DB-5 (Enlarged Section, ECD)

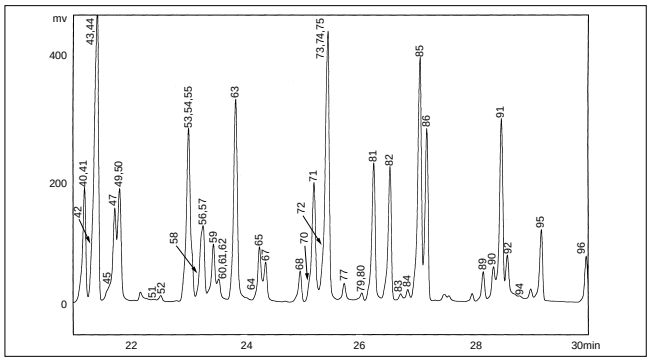


Fig. 1.1.15 Chromatogram for 124 Pesticides Using DB-5 (Enlarged Section, ECD)

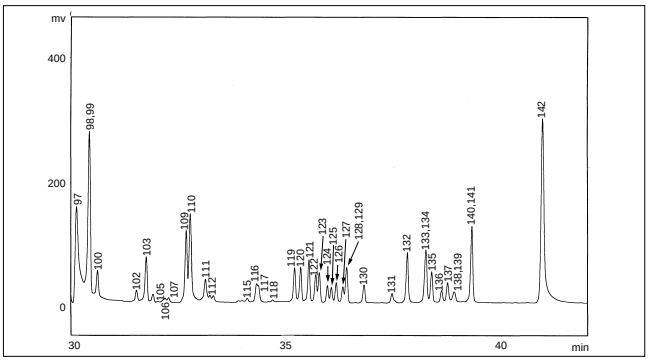


Fig. 1.1.16 Chromatogram for 124 Pesticides Using DB-5 (Enlarged Section, ECD)

Table 1.1.4 Relative Retention Times for 124 Pesticides Using DB-5 (with EPN assumed to be 30 minutes)

Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time
1	DCIP	4.968	37	methiocarb	20.705	73	pretilachlor	25.156	108	amitraz	31.899
2	methamidophos	6.455	38	esprocarb	20.833	74	dieldrin	25.179	109	cyhalothrin-2	32.157
3	dichlorvos	6.816	39	pirimifos-methyl	20.833	75	DDE(p,p')	25.179	110	fenarimol	32.266
4	EPTC	8.777	40	dimethylvinphos(E)	20.916	76	difenzoquat methyl sulfate	25.291	111	acrinathrin	32.634
5	propamocarb	9.258	41	dichlofluanid	20.997	77	myclobutanil	25.389	112	pyraclofos	32.806
6	butylate	9.999	42	thiobencarb	21.119	78	flusilazole	25.532	113	bitertanol-1	33.389
7	acephate	10.081	43	malathion	21.214	79	cyproconazole-1	25.854	114	bitertanol-2	33.588
8	isoprocarb	12.224	44	aldrin	21.259	80	cyproconazole-2	25.872	115	permethrin-1	33.589
9	fenobucarb	13.701	45	metolachlor	21.299	81	endrin	25.984	116	permethrin-2	33.841
10	ethoprophos	14.262	46	diethofencarb	21.357	82	chlorphenapyr	26.148	117	pyridaben	33.841
11	chlorpropham	14.594	47	dimethylvinphos(Z)	21.428	83	chlorobenzilate	26.369	118	inabenfide	34.178
12	bendiocarb	15.057	48	fenthion	21.459	84	fensulfothion	26.475	119	cafenstrole	34.670
13	trifluralin	15.288	49	parathion	21.581	85	DDD(p,p')	26.709	120	cyfluthrin-1	34.799
14	cadusafos	15.353	50	chlorpyrifos	21.581	86	DDT(o,p')	26.846	121	cyfluthrin-2	34.983
15	BHC(α)	15.696	51	fosthiazate-1	22.091	87	mepronil	27.168	122	cyfluthrin-3	35.146
16	thiometon	15.931	52	fosthiazate-2	22.191	88	lenacil	27.800	123	cyfluthrin-4	35.222
17	dimethoate	16.185	53	chlorfenvinphos(α)	22.776	89	edifenphos	27.802	124	cypermethrin-1	35.422
18	dimethipin	16.568	54	heptachlor epoxide	22.829	90	propiconazole-1	27.940	125	halfenprox	35.493
19	BHC(β)	16.749	55	pendimethalin	22.829	91	DDT(p,p')	28.111	126	cypermethrin-2	35.620
20	BHC(γ)	17.012	56	pyrifenox-1	22.958	92	propiconazole-2	28.178	127	cypermethrin-3	35.751
21	terbufos	17.285	57	captan	23.038	93	tebuconazole	28.537	128	cypermethrin-4	35.861
22	diazinon	17.840	58	isofenphos	23.133	94	thenylchlor	28.537	129	flucythrinate-1	35.861
23	BHC(δ)	17.940	59	chlorfenvinphos(β)	23.162	95	captafol	28.810	130	flucythrinate-2	36.255
24	tefluthrin	18.314	60	phenthoate	23.271	96	pyributicarb	29.518	131	pyrimidifen	36.904
25	etrimfos	18.421	61	triadimenol	23.271	97	acetamiprid	29.697	132	fenvalerate-1	37.265
26	ethiofencarb	18.807	62	quinalphos	23.271	98	EPN	30.000	133	fenvalerate-2	37.684
27	pirimicarb	18.807	63	chinomethionat	23.592	99	bifenthrin	30.159	134	fluvalinate-1	37.684
28	dimethenamid	19.250	64	paclobutrazol	23.884	100	dicofol	30.248	135	fluvalinate-2	37.806
29	metribuzin	19.305	65	pyrifenox-2	23.976	101	tebufenpyrad	30.490	136	difenoconazole-1	38.042
30	bentazone	19.515	66	vamidothion	23.986	102	furametpyr	31.048	137	difenoconazole-2	38.179
31	parathion-methyl	19.593	67	trichlamide	24.027	103	phosalone	31.306	138	deltamethrin-1	38.348
32	tolclophos-methyl	19.692	68	butamifos	24.610	104	pyriproxyfen	31.501	139	pyrazoxyfen	38.348
33	carbaryl	19.743	69	flutolanil	24.748	105	mefenacet	31.681	140	deltamethrin-2	38.737
34	heptachlor	19.876	70	tricyclazole	24.772	106	cyhalofop-butyl	31.707	141	tralomethrin	38.737
35	alachrol	19.906	71	prothiofos	24.892	107	cyhalothrin-1	31.765	142	imibenconazole	40.352
36	fenitrothion	20.705	72	fludioxonil	24.997						

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (5) - GC

■DB-1301 Separation

Table 1.1.5 Analytical Conditions

Instrument	: GC-17AAFwFtE ver.3, AOC-20i	Detector	: FTD-17, ECD-17
Column	: DB-1301 (30m × 0.25mm I.D. df=0.25μm)	Detector temperature	: 300°C
Column temperature	: 50°C(1min)-20°C/min-120°C -5°C/min-280°C(15min)	Injection inlet temperature	: 280°C
		Injection method	: High Pressure Splitless (300kPa, 1min)
Carrier Gas	: He, 150kPa	Injection volume	: 2μL

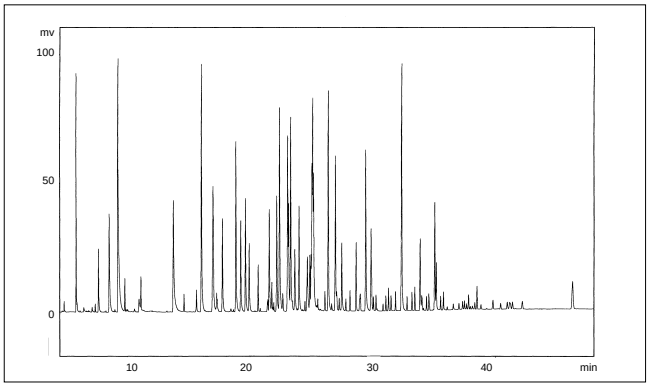


Fig. 1.1.17 Chromatogram for 124 Pesticides Using DB-1301
(Full Chromatogram, FTD)

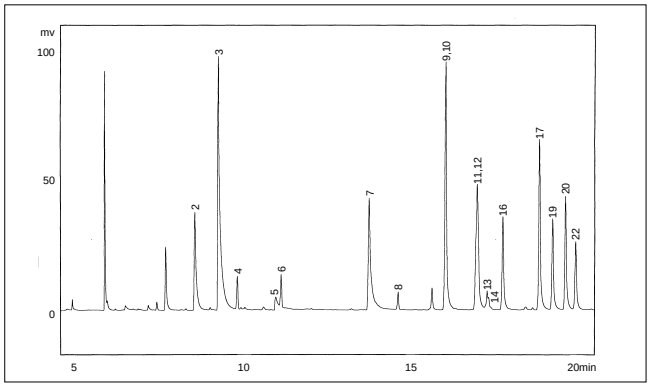


Fig. 1.1.18 Chromatogram for 124 Pesticides Using DB-1301
(Enlarged Section, FTD)

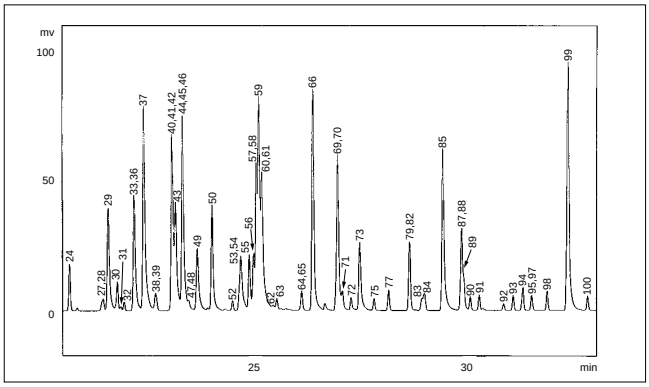


Fig. 1.1.19 Chromatogram for 124 Pesticides Using DB-1301
(Enlarged Section, FTD)

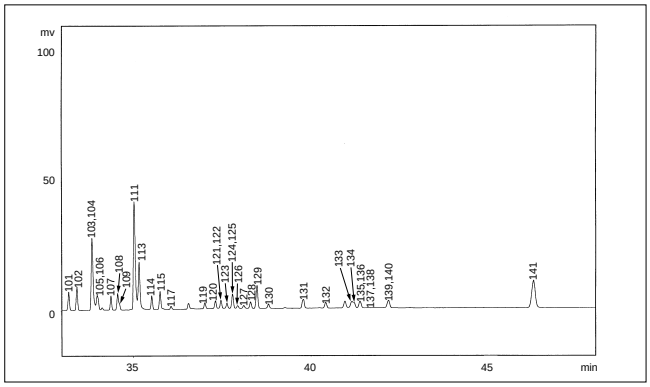


Fig. 1.1.20 Chromatogram for 124 Pesticides Using DB-1301
(Enlarged Section, FTD)

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (6) - GC

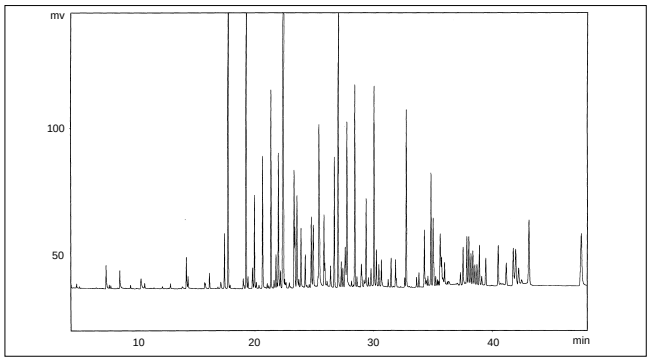


Fig. 1.1.21 Chromatogram for 124 Pesticides Using DB-1301 (Full Chromatogram, ECD)

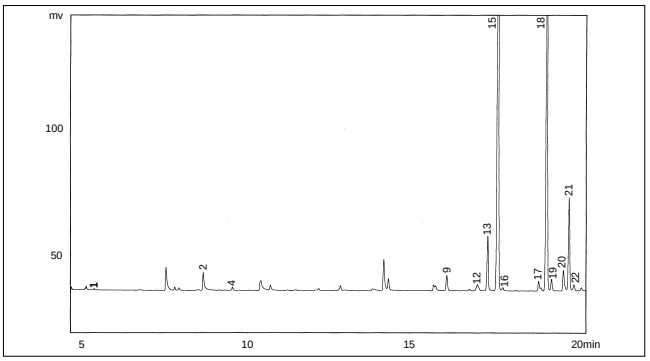


Fig. 1.1.22 Chromatogram for 124 Pesticides Using DB-1301 (Enlarged Section, ECD)

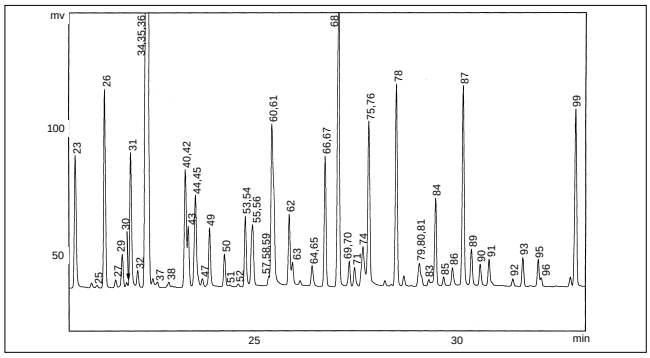


Fig. 1.1.23 Chromatogram for 124 Pesticides Using DB-1301 (Enlarged Section, ECD)

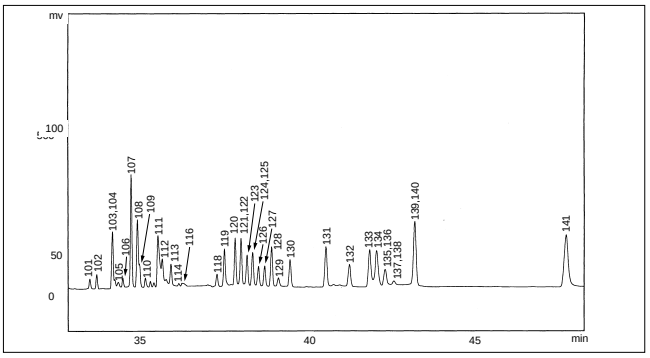


Fig. 1.1.24 Chromatogram for 124 Pesticides Using DB-1301 (Enlarged Section, ECD)

Table 1.1.6 Relative Retention Times for 124 Pesticides Using DB-1301 (with EPN assumed to be 30 minutes)

Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time
1	DCIP	4.800	37	pirimifos-methyl	20.788	72	difenzoquat methyl sulfate	25.299	107	cyhalothrin-2	31.900
2	dichlorvos	7.909	38	thiobencarb	21.041	73	vamidothion	25.484	108	acrinathrin	32.075
3	methamidophos	8.562	39	carbaryl	21.041	74	endrin	25.512	109	fenarimol	32.107
4	EPTC	9.092	40	chlorpyrifos	21.402	75	flutolanil	25.799	110	permethrin-1	32.237
5	propamocarb	10.162	41	methiocarb	21.402	76	DDT(o,p')	25.799	111	pyraclofos	32.510
6	butylate	10.309	42	dimethylvinphos(E)	21.402	77	flusilazole	26.116	112	permethrin-2	32.552
7	acephate	12.757	43	fenitrothion	21.473	78	chlorobenzilate	26.218	113	acetamiprid	32.636
8	isoprocarb	13.566	44	dichlofluanid	21.631	79	myclobutanil	26.569	114	pyridaben	32.960
9	ethoprophos	14.881	45	malathion	21.631	80	DDD(p,p')	26.569	115	bitertanol-1	33.181
10	fenobucarb	14.881	46	fenthion	21.631	81	dicofol	26.569	116	inabenfide	33.059
11	chlorpropham	15.757	47	metolachlor	21.761	82	tricyclazole	26.569	117	bitertanol-2	33.462
12	cadusafos	15.757	48	diethofencarb	21.761	83	cyproconazole	26.838	118	halfenprox	34.121
13	trifluralin	16.034	49	dimethylvinphos(Z)	21.946	84	chlorfenapyr	26.892	119	cyfluthrin-1	34.347
14	bendiocarb	16.074	50	parathion	22.205	85	fensulfothion	27.289	120	cyfluthrin-2	34.627
15	BHC(α)	16.324	51	heptachlor epoxide	22.408	86	DDT(p,p')	27.474	121	cyfluthrin-3	34.770
16	thiometon	16.468	52	pyrifenox-1	22.715	87	edifenphos	27.698	122	cypermethrin-1	34.770
17	terbufos	17.484	53	chlorfenvinphos(α)	22.896	88	mepronil	27.698	123	cyfluthrin-4	34.927
18	BHC(γ)	17.711	54	pendimethalin	22.896	89	fludioxonil	27.737	124	cafenstrole	35.073
19	diazinon	17.851	55	fosthiazate	23.081	90	propiconazole-1	27.889	125	cypermethrin-2	35.073
20	dimethoate	18.207	56	fosthiazate	23.177	91	propiconazole-2	28.083	126	cypermethrin-3	35.207
21	tefluthrin	18.356	57	quinalphos	23.238	92	thenylchlor	28.613	127	cypermethrin-4	35.365
22	etrimfos	18.490	58	chinomethionat	23.238	93	pyributicarb	28.820	128	flucythrinate-1	35.541
23	dimethipin	18.990	59	isofenphos	23.292	94	lenacil	29.030	129	pyrimidifen	35.713
24	pirimicarb	19.173	60	chlorfenvinphos(β)	23.355	95	captafol	29.217	130	flucythrinate-2	36.010
25	heptachlor	19.481	61	phenthoate	23.355	96	bifenthrin	29.217	131	fenvalerate-1	36.923
26	BHC(β)	19.661	62	pyrifenox-2	23.601	97	tebuconazole	29.217	132	fenvalerate-2	37.502
27	dimethenamid	19.906	63	captan	23.682	98	tebufenpyrad	29.553	133	fluvalinate-1	38.187
28	ethiofencarb	19.906	64	triadimenol	24.223	99	EPN	30.000	134	difenoconazole-1	38.223
29	tolclophos-methyl	20.013	65	trichlamide	24.223	100	pyriproxyfen	30.428	135	difenoconazole-2	38.395
30	metribuzin	20.211	66	prothiofos	24.467	101	furametpyr	30.799	136	fluvalinate-2	38.395
31	bentazone	20.285	67	DDE(p,p')	24.467	102	amitraz	31.014	137	deltamethrin-1	38.627
32	alachrol	20.359	68	dieldrin	24.765	103	phosalone	31.407	138	pyrazoxyfen	38.627
33	esprocarb	20.575	69	paclobutrazol	25.008	104	cyhalothrin-1	31.407	139	deltamethrin-2	39.139
34	BHC(δ)	20.575	70	butamifos	25.008	105	mefenacet	31.541	140	tralomethrin	39.139
35	aldrin	20.575	71	pretilachlor	25.110	106	cyhalofop-butyl	31.541	141	imibenconazole	42.920
36	parathion-methyl	20.575									

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (7) - GC

■DB-17 Separation

Table 1.1.7 Analytical Conditions

Instrument	: GC-17AAFwFtE ver.3, AOC-20i	Detector	: FTD-17, ECD-17
Column	: DB-17(30m × 0.25mm I.D. df=0.25µm)	Detector temperature	: 300°C
Column temperature	: 50°C(1min)-20°C/min - 120°C - 5°C/min-280°C(15min)	Injection inlet temperature	: 280°C
		Injection method	: High Pressure Splitless (300kPa, 1min)
Carrier Gas	: He, 150kPa-5kPa/min-350kPa	Injection volume	: 2µL

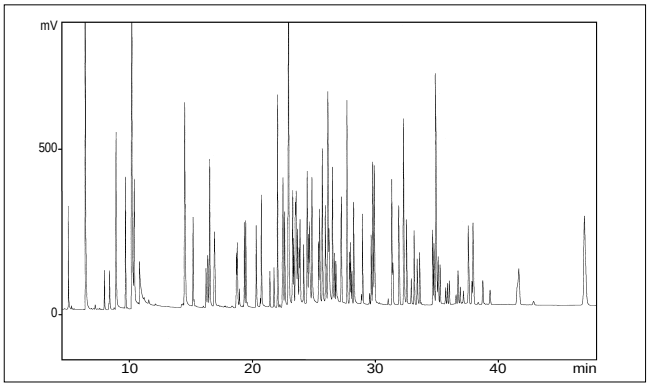


Fig. 1.1.25 Chromatogram for 124 Pesticides Using DB-17 (Full Chromatogram, FTD)

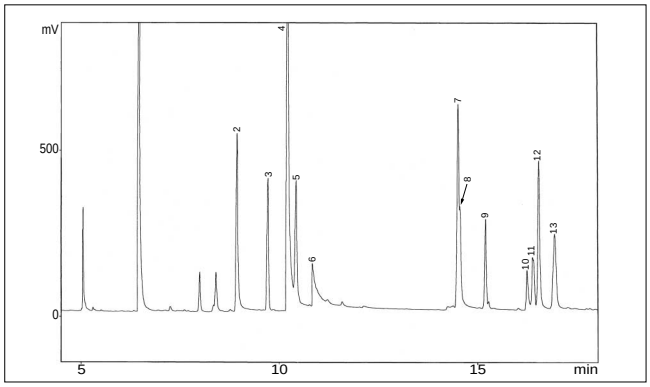


Fig. 1.1.26 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, FTD)

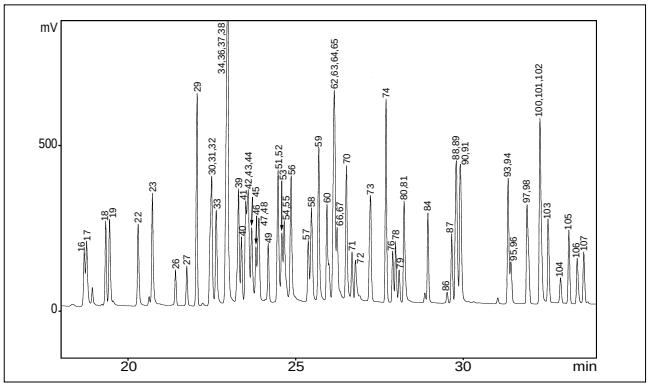


Fig. 1.1.27 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, FTD)

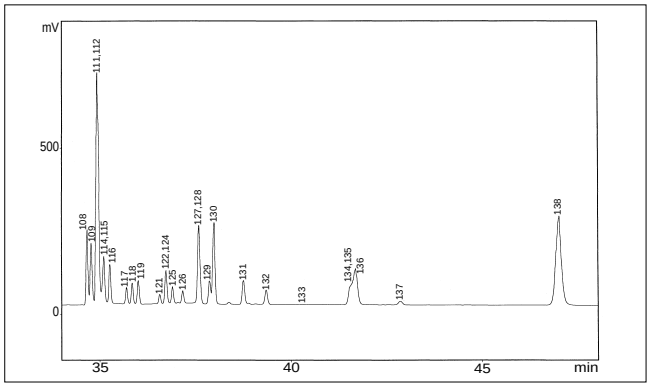


Fig. 1.1.28 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, FTD)

1.1 Analysis of 124 Pesticides With Capillary GC-FTD and GC-ECD (8) - GC

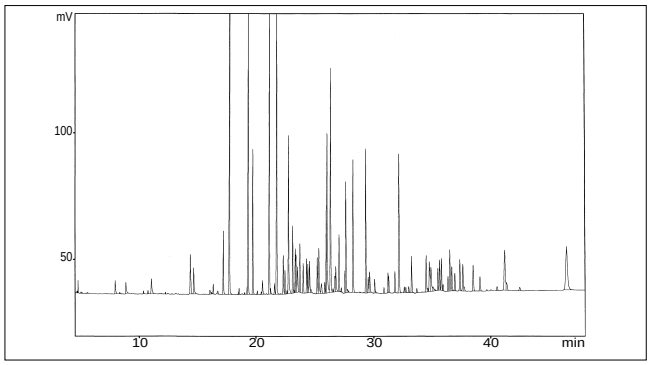


Fig. 1.1.29 Chromatogram for 124 Pesticides Using DB-17 (Full Chromatogram, ECD)

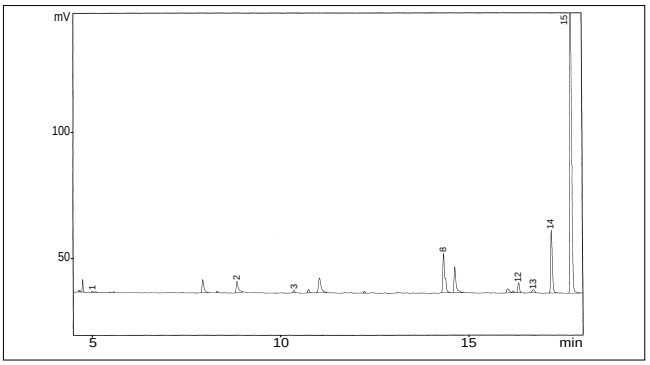


Fig. 1.1.30 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, ECD)

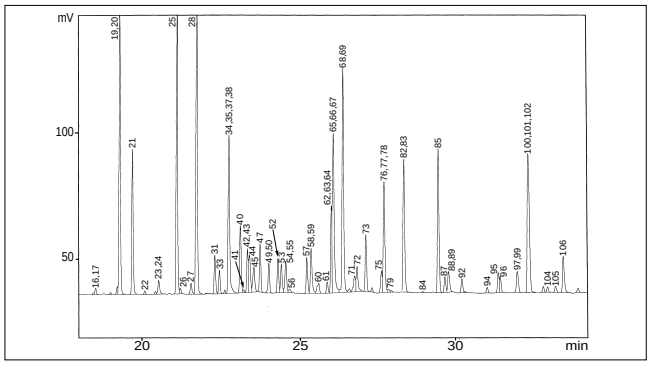


Fig. 1.1.31 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, ECD)

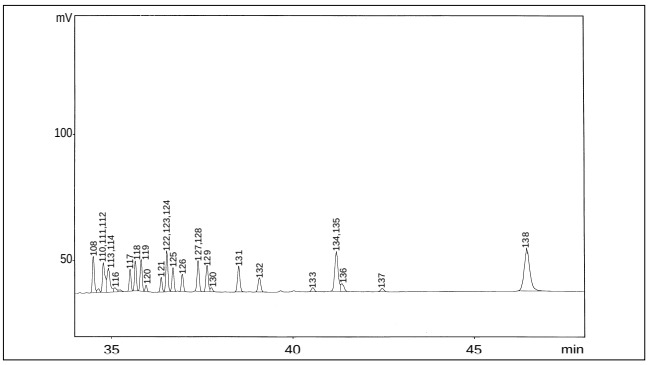


Fig. 1.1.32 Chromatogram for 124 Pesticides Using DB-17 (Enlarged Section, ECD)

Table 1.1.8 Relative Retention Times for 124 Pesticides Using DB-17 (with EPN assumed to be 30 minutes)

Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time	Peak No.	Compound	Relative Retention Time
1	DCIP	5.199	36	pirimifos-methyl	21.290	71	flutoluanil	24.756	105	furametpyr	30.772
2	dichlorvos	8.246	37	metolachlor	21.300	72	captan	24.990	106	phosalone	31.003
3	EPTC	8.920	38	bentazone	21.340	73	chlorphenapyr	25.288	107	pyriproxyfen	31.176
4	methamidophos	9.459	39	diethofencarb	21.552	74	flusilazole	25.288	108	fenarimol	32.184
5	butylate	9.652	40	chlorpyrifos	21.598	75	chlorobenzilate	25.676	109	bitertanol-1	32.280
6	propamocarb	9.959	41	thiobencarb	21.692	76	myclobutanil	25.777	110	permethrin-1	32.411
7	acephate	13.444	42	parathion	21.807	77	endrin	25.777	111	acetamiprid	32.418
8	trifluralin	13.452	43	fenitrothion	21.807	78	cyproconazole	25.892	112	pyraclofos	32.418
9	isoprocarb	14.074	44	dimethylvinphos(E)	21.860	79	cyproconazole	25.973	113	permethrin-2	32.566
10	fenobucarb	15.193	45	malathion	21.977	80	difenzoquat methyl sulfate	26.075	114	pyridaben	32.566
11	chlorpropham	15.193	46	carbaryl	22.107	81	vamidothion	26.224	115	bitertanol-2	32.566
12	ethoprophos	15.292	47	dichlofluanid	22.176	82	DDT(o,p')	26.365	116	mefenacet	32.732
13	cadusafos	15.635	48	methiocarb	22.186	83	DDD(p,p')	26.365	117	cyfluthrin-1	33.115
14	tefluthrin	16.061	49	pendimethalin	22.410	84	fludioxonil	26.870	118	cyfluthrin-2	33.253
15	BHC(α)	16.593	50	heptachlor epoxide	22.381	85	DDT(p,p')	27.341	119	cyfluthrin-3	33.399
16	thiometon	17.315	51	fenthion	22.698	86	mepronil	27.409	120	halfenprox	33.523
17	terbufos	17.378	52	dimethylvinphos(Z)	22.698	87	propiconazole-2	27.541	121	cypermethrin-1	33.924
18	bendiocarb	17.923	53	chlorfenvinphos(α)	22.797	88	propiconazole-1	27.658	122	cypermethrin-2	34.074
19	diazinon	18.012	54	triadimenol	22.898	89	fensulfothion	27.658	123	inabenfide	34.074
20	BHC(γ)	18.082	55	trichlamide	22.928	90	tricyclazole	27.775	124	flucythrinate-1	34.074
21	BHC(β)	18.448	56	isofenphos	23.045	91	tebuconazole	27.775	125	cypermethrin-3	34.235
22	etrimfos	18.816	57	chlorfenvinphos(β)	23.536	92	bifenthrin	28.054	126	flucythrinate-2	34.481
23	dimethoate	19.215	58	paclobutrazol	23.615	93	tebufenpyrad	28.095	127	cafenstrole	34.863
24	heptachlor	19.215	59	pyrifenox-1	23.653	94	edifenphos	29.113	128	fluvalinate-1	34.891
25	BHC(σ)	19.750	60	quinalphos	23.833	95	acrinathrin	29.176	129	fluvalinate-2	35.129
26	dimethenamid	19.830	61	pretilachlor	24.118	96	thenylchlor	29.176	130	pyrimidifen	35.227
27	tolclophos-methyl	20.146	62	prothiofos	24.262	97	pyributicarb	29.629	131	fenvalerate-1	35.956
28	aldrin	20.325	63	pyrifenox-2	24.262	98	lenacil	29.638	132	fenvalerate-2	36.499
29	pirimicarb	20.447	64	chinomethionat	24.262	99	cyhalothrin-1	29.656	133	deltamethrin-1	37.894
30	ethiofencarb	20.829	65	fosthiazate	24.274	100	cyhalothrin-2	30.000	134	deltamethrin-2	38.522
31	parathion-methyl	20.875	66	phenthoate	24.297	101	captafol	30.000	135	tralomethrin	38.522
32	esprocarb	20.875	67	fosthiazate	24.343	102	EPN	30.000	136	difenoconazole	38.619
33	alachrol	20.976	68	DDE(p,p')	24.501	103	amitraz	30.211	137	pyrazoxyfen	39.730
34	metribuzin	21.290	69	dieldrin	24.577	104	cyhalofop-butyl	30.535	138	imibenconazole	43.593
35	dimethipin	21.290	70	butamifos	24.608						

1.2 Analysis of Organophosphorus Pesticide Residue in Agricultural Products (1) - GC

Analysis Based on Standards for Foods and Additives Specified in Japan's Food Sanitation Law (Notice 370 Issued by Japan's Ministry of Health,Labour and Welfare)

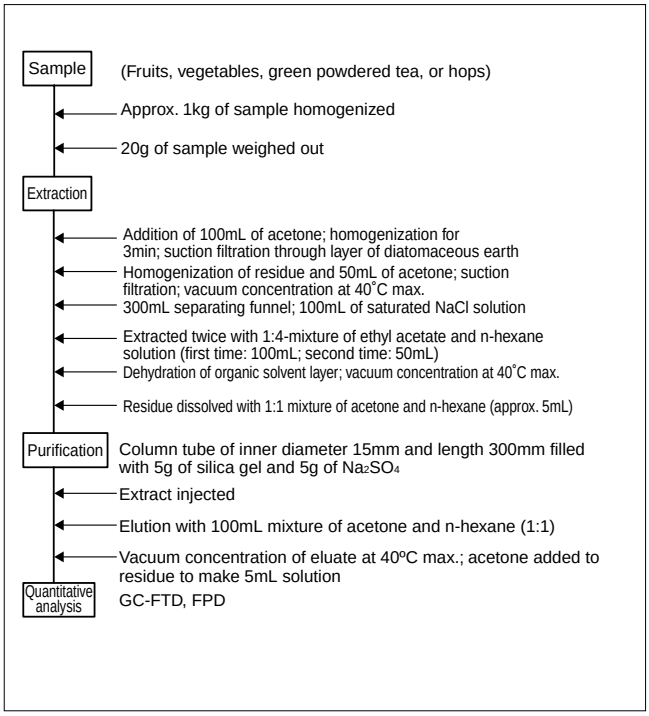
Explanation

As a result of the diversification of foods and improvements in the ways foods are transported and stored, a wide variety of food products are imported from all over the world and eaten as part of our daily diet. There are, however, many reported cases of excessive levels of pesticide residue being found in imported foods, particularly imported vegetables, and the safety of imported vegetables has become an issue of some concern. The analysis of organophosphorus pesticide based on the standards for foods and additives specified in Japan's Food Sanitation Law (Japan's Ministry of Health,Labour and Welfare: Notice 370, D, item (6)) is described here as an example.

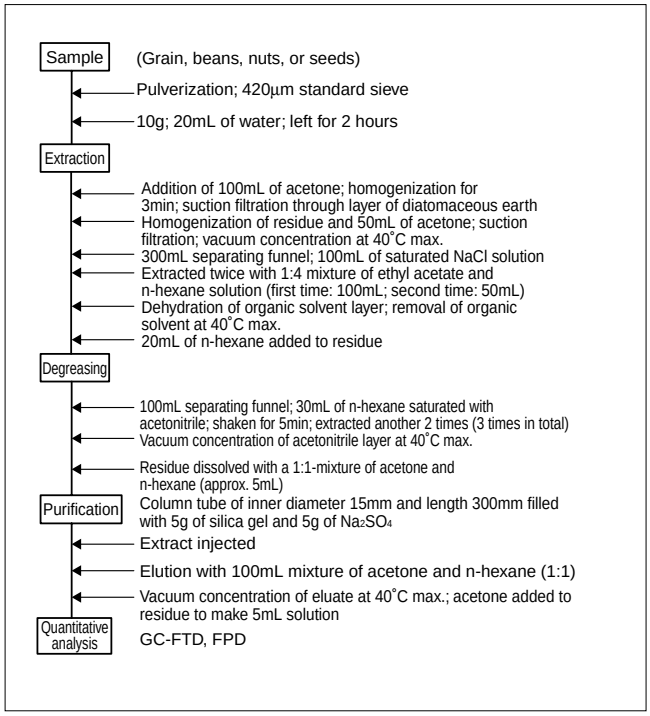
The analysis method varies with the sample; samples are categorized into three groups that each have different analysis methods: fruits, vegetables, green powdered tea, and hops; grain, beans, nuts, and seeds; and teas other than green powdered tea. The sample processing methods for the first two groups are described below.

Pretreatment

(Fruits, vegetables, green powdered tea, and hops)



(Grain, beans, nuts, and seeds)



1.2 Analysis of Organophosphorus Pesticide Residue in Agricultural Products (2) - GC

Fig. 1.2.1 shows a chromatogram of 0.1mg/L standard organophosphorus pesticide solution. Analysis was performed after adding standard organophosphorus pesticide solution.
Fig. 1.2.2 to 1.2.4 show the chromatograms of apples, spinach, and soybeans.

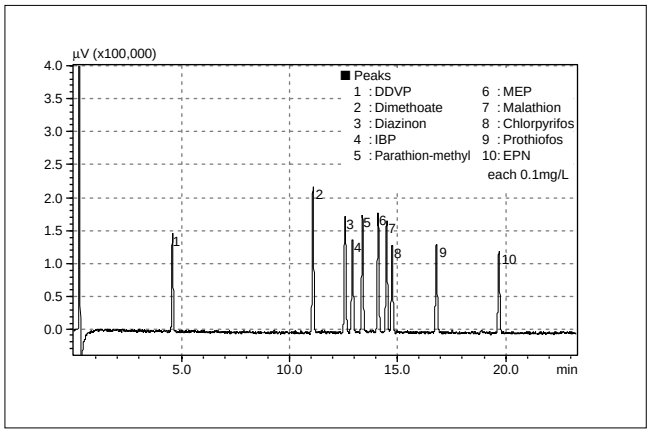


Fig. 1.2.1 Chromatogram of standard organophosphorus pesticide solution (0.1mg/L)

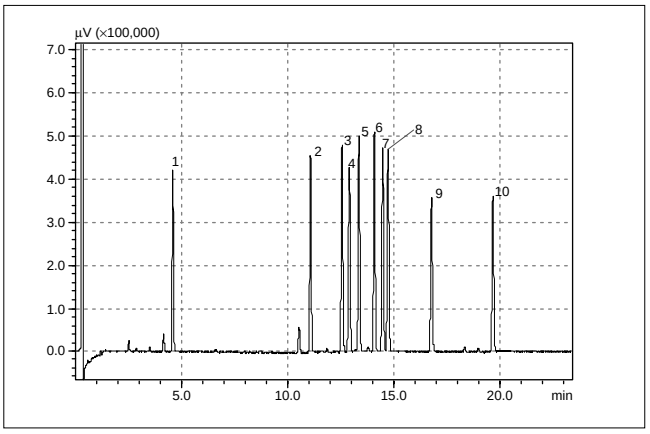


Fig. 1.2.2 Chromatogram of apples (0.05μg/g standard pesticide solution added)

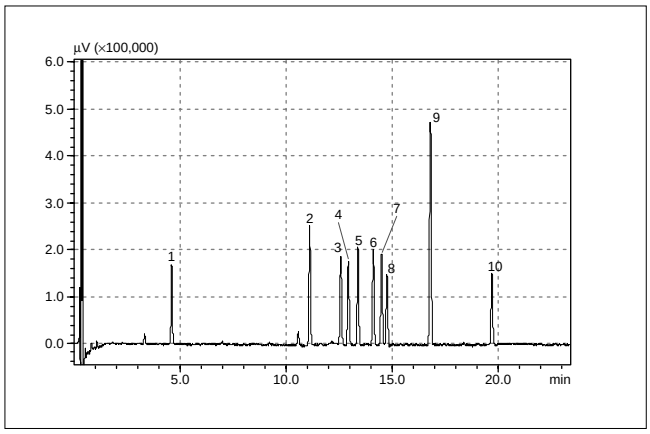


Fig. 1.2.3 Chromatogram of spinach (0.05μg/g standard pesticide solution added)

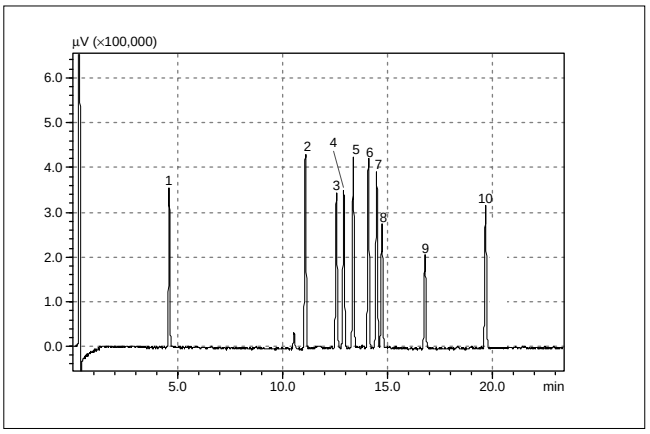


Fig. 1.2.4 Chromatogram of soybeans (0.1μg/g standard pesticide solution added)

■Analytical Conditions

Instrument : GC-2010AF, FPD-2010, AOC-20i
Column : Rtx-1 (15m × 0.53mm I.D., df = 1.5μm)
Column Temp. : 80˚C(1min)-8˚C/min-250˚C(5min)
Carrier Gas : He, 46kPa (16.5mL/min, 120cm/s, constant-velocity mode)
Detector : FPD-2010 (P Filter)
Inj. Temp. : 230˚C
Det. Temp : 280˚C
Injection method : Splitless (1min)
Injection volume: 1μL

1.3 Analysis of Organophosphorus Pesticide Residue in Agricultural Products (1) - GC

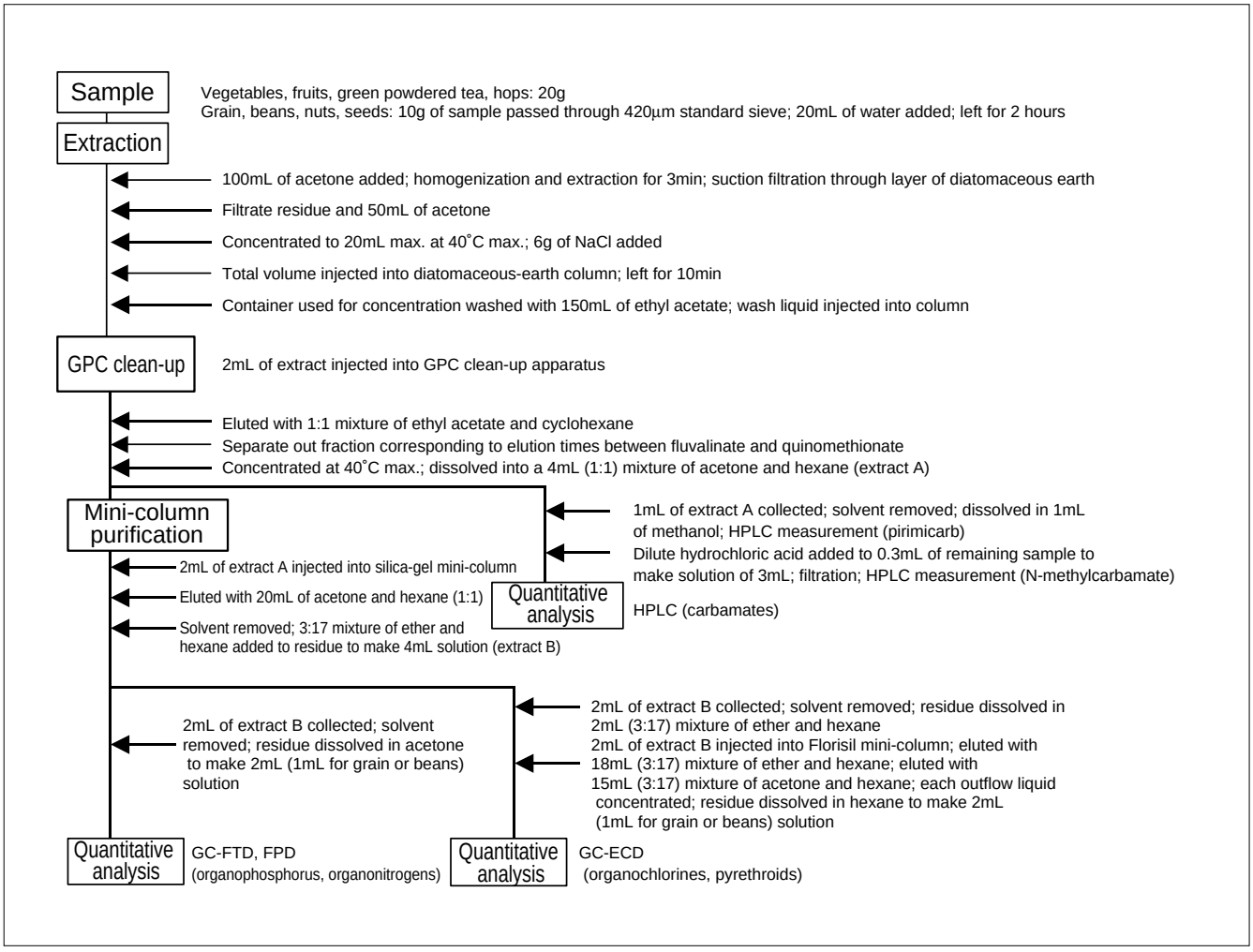
Analysis Based on the Rapid Analysis Method Specified in Japan's Food Sanitation Law (Notices 43, 44, and 45 Issued by the Environmental Health Bureau's Food Chemistry Division in 1997)

■Explanation

In 1997, Japan's Ministry of Health, Labour and Welfare issued notification of a pesticide-residue rapid analysis method as a simple and quick way for screening many pesticide residues. With this method, the same analysis method can be used for many different agricultural products and pesticides and some of the pretreatment operations can be automated using the GPC method. The pesticides are analyzed in groups, such as chlorine, phosphorus, and nitrogen, using GC-ECD, GC-FPD, and GC-FTD. If, however, pesticide residue with a concentration exceeding approximately 50% of the regulated value is detected using the rapid analysis method, quantitative measurement must be carried out as stipulated by the corresponding notification. The analysis of organophosphorus pesticide is described as an example.

■Pretreatment

Extraction with acetone is carried out on the sample and, after redissolving in ethyl acetate with a diatomaceous-earth column, GPC clean-up is performed and the sample is purified with a silica-gel mini-column. Organophosphorus pesticides are analyzed with GC-FPD or GC-FTD after concentration. Carbamate pesticides are analyzed by taking a sample of the solution after performing GPC clean-up and analyzing the sample in this state or after diluting with hydrochloric acid. Organochlorine or pyrethroid pesticides are investigated by performing analysis after refining first with silica gel and then with a Florisil mini-column.



1.3 Analysis of Organophosphorus Pesticide Residue in Agricultural Products (2) - GC

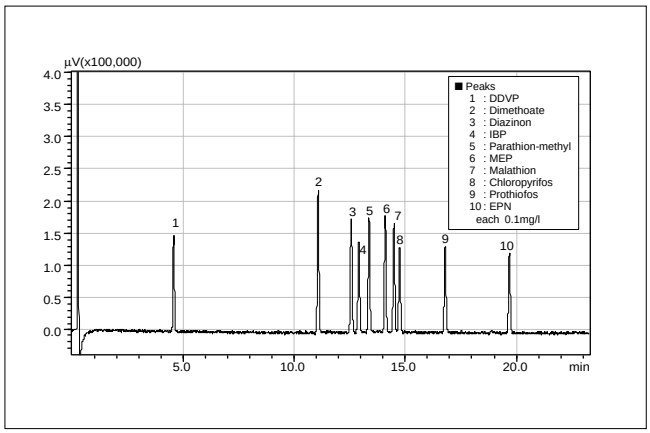


Fig. 1.3.1 Chromatogram of standard organophosphorus pesticides solution (0.1mg/L)

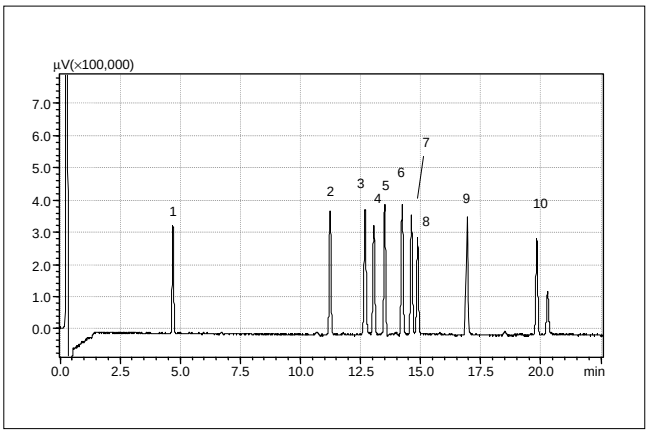


Fig. 1.3.2 Chromatogram of processed spinach liquid with 0.05μg/g standard pesticide solution added

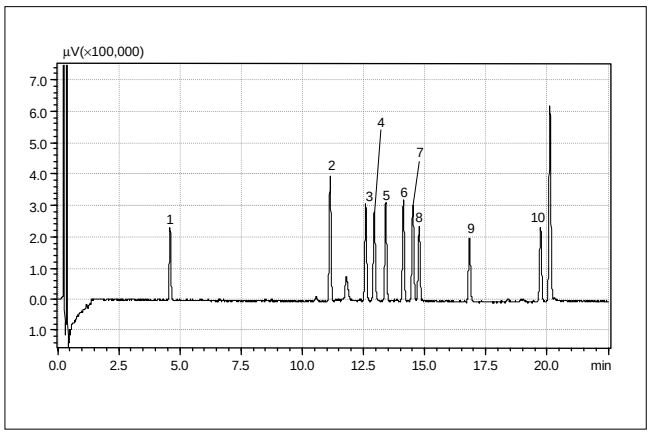


Fig. 1.3.3 Chromatogram of processed soybean liquid with 0.1μg/g standard pesticide solution added

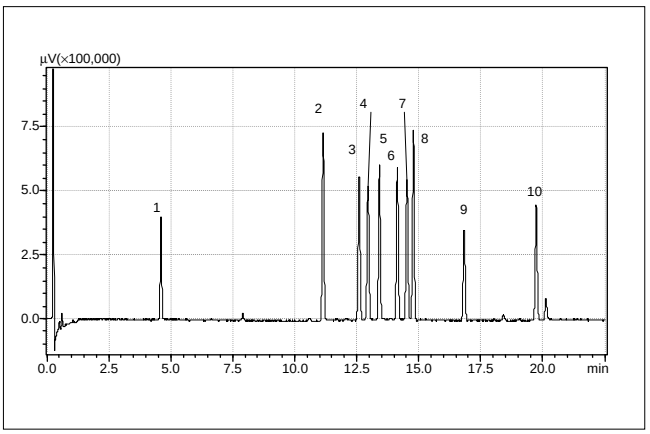


Fig. 1.3.4 Chromatogram of processed rice liquid with 0.1μg/g standard pesticide solution added

[References]

Handbook for Food Sanitation Laws, 2003 Edition, Shinnippon-hoki Publishing Co., Ltd., (2002)

■Analytical Conditions

Instrument : GC-2010AF, FPD-2010, AOC-20i
Column : Rtx-1 (15m × 0.53mm I.D., df = 1.5μm)
Column Temp. : 80˚C(1min)-8˚C/min-250˚C(5min)
Carrier Gas : He, 46kPa (16.5mL/min, 120cm/s, constant-velocity mode)
Detector : FPD-2010 (P Filter)
Inj. Temp. : 230˚C
Det. Temp. : 280˚C
Injection method : Splitless (1min)
Injection volume : 1μL

1.4 Analysis of Organonitrogen and Pyrethroid Pesticide Residue in Agricultural Products (1) - GC

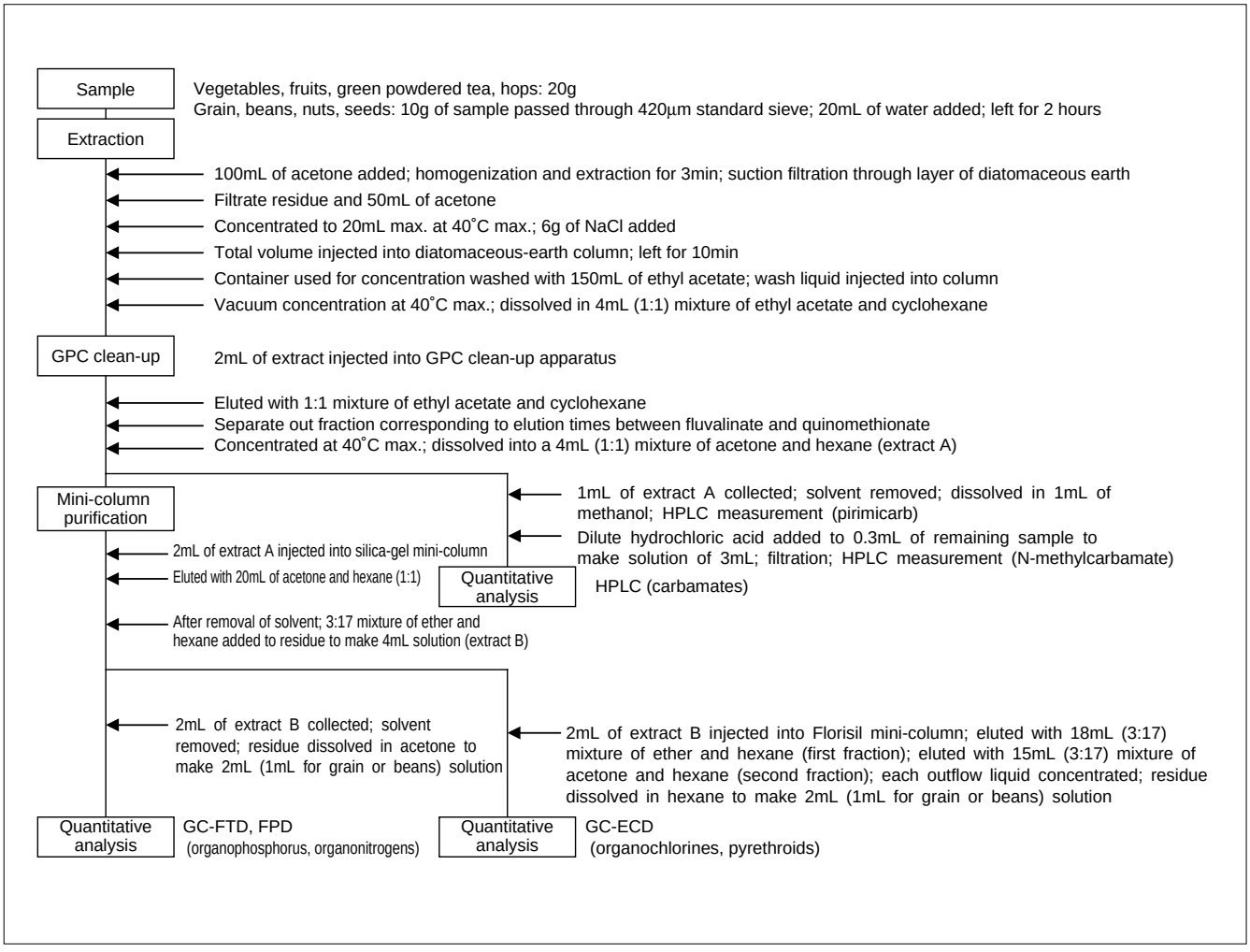
■Explanation

The analysis of organonitrogen and pyrethroid pesticides using the rapid analysis method is described here. Fig. 1.4.1 and 1.4.2 show chromatograms of standard organophosphorus and standard organonitrogen pesticide solutions analyzed under the same conditions. Both organophosphorus and organonitrogen pesticides can be detected with GC-FTD. Also, in the pretreatment for the rapid analysis method, both pesticides are eluted into the same fraction. Fig. 1.4.3 shows a chromatogram of processed soy bean liquid with 13 organophosphorus and 14 organonitrogen pesticides added.

Fig. 1.4.4 shows a chromatogram of a standard pyrethroid pesticide solution. Separation and quantitative analysis can be difficult for pyrethroid pesticides as there are often many isomers within a standard product. Consideration is also required for substances that convert their forms at the GC injector inlet (for example, deltamethrin changes to tralomethrin). Fig. 1.4.5 shows a chromatogram of processed spinach liquid with a standard pyrethroid pesticide added.

■Pretreatment

(Pretreatment for Pesticide-residue Rapid Analysis Method)



1.4 Analysis of Organonitrogen and Pyrethroid Pesticide Residue in Agricultural Products (2) - GC

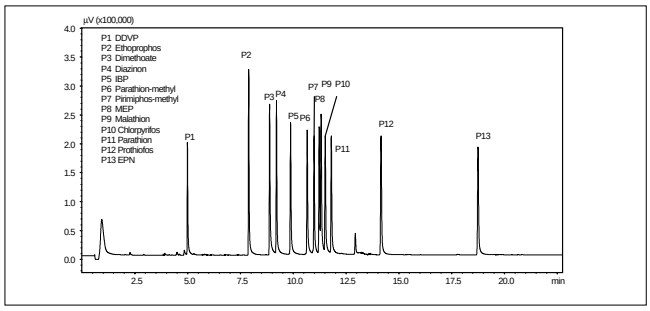


Fig. 1.4.1 Chromatogram of standard organophosphorus pesticides solution obtained using GC-FTD

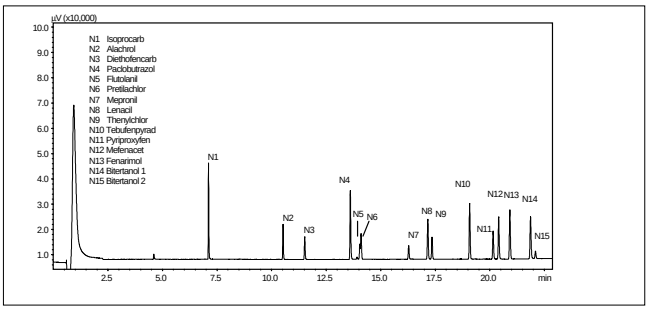


Fig. 1.4.2 Chromatogram of standard organonitrogen pesticides solution obtained using GC-FTD

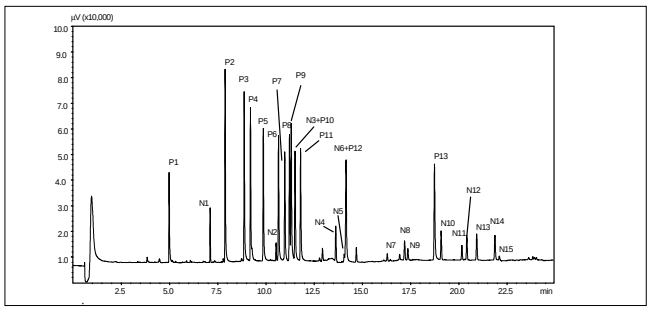


Fig. 1.4.3 Chromatogram of processed soy bean solution obtained using GC-FTD (13 organophosphorus and 14 organonitrogen pesticides: 0.2 to 0.5μg/g added)

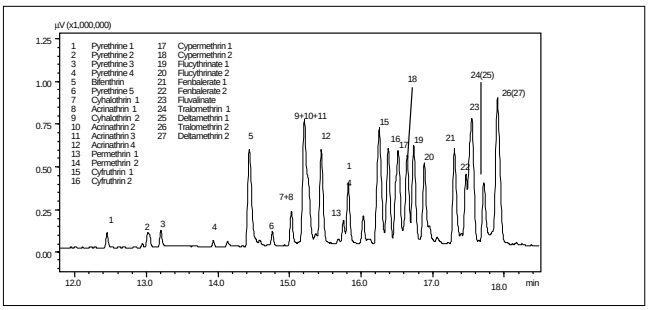


Fig. 1.4.4 Chromatogram of standard pyrethroid pesticides solution obtained using GC-ECD (1mg/L)

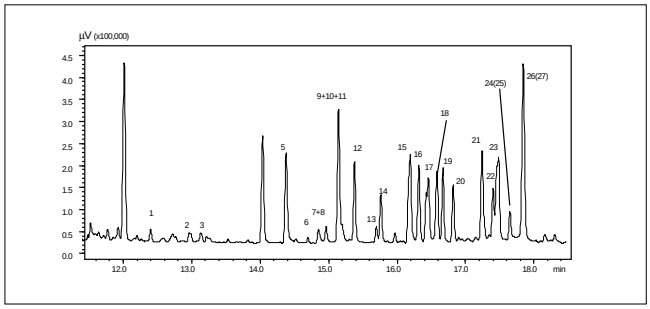


Fig. 1.4.5 Chromatogram of processed spinach liquid obtained using GC-ECD (first fraction, 0.1μg/g pesticide added)

■Analytical Conditions 1

Instrument : GC-2010AF, FPD-2010, AOC-20i, GCsolution
Column : BPX5 (30m × 0.25mm I.D., df = 0.25μm)
Column Temp. : 80˚C(1min)-20˚C/min-190˚C-5˚C/min-280˚C(5min)
Carrier Gas : He, 143kPa (2.4mL/min, 45cm/s, constant-velocity mode)
Detector : FTD-2010
Inj. Temp. : 250˚C
Det. Temp. : 280˚C
Injection method : High-pressure, splitless (300kPa, 1min)
Injection volume : 1μL

■Analytical Conditions 2

Instrument : GC-17A, ECD-17, AOC-20i, GCsolution
Column : ZB-1 (30m × 0.25mm I.D., df = 0.25μm)
Column Temp. : 50˚C(1min)-25˚C/min-175˚C-10˚C/min-300˚C(4min)
Carrier Gas : He, 150kPa (1.7mL/min, constant-pressure mode)
Detector : ECD-17
Inj. Temp. : 280˚C
Det. Temp. : 310˚C
Injection method : High-pressure, splitless (300kPa, 1min)
Injection volume : 1μL

1.5 Analysis of Phoxim in Agricultural Products Using Short Capillary Column - GC

■Explanation

Phoxim is an organophosphorus insecticide that has a wide range of effects. It is also known, however, to decompose easily in analysis when exposed to high temperatures in, for example, the injection inlet or column. In the example presented here, a short capillary column of length 5m is used in order to minimize the level of decomposition of phoxim in GC analysis and improve the level of separation from other organophosphorus pesticides.

■Analysis Using the Notified Method

Fig. 1.5.1 shows the chromatogram obtained for a standard mixture of organophosphorus pesticides under the notified conditions for phoxim (using a wide-bore column of inner diameter 0.53mm and length 10m). The separation of pirimifos-methyl, malathion, parathion, chlorpyrifos, and phoxim is insufficient.

■Analysis Using Short Capillary Column

Fig. 1.5.2 shows the chromatogram obtained for the same standard mixture using a short capillary column of inner diameter 0.22mm and length 5m (high-pressure splitless analysis at 350kPa). It can be seen that there is better separation for phoxim. Also, using a short column makes it possible to elute phoxim at the relatively low temperature of 130ºC and so we can expect a reduction in the level of decomposition of phoxim in the column. Fig. 1.5.3 shows a chromatogram for soybean to which phoxim was added to a concentration of approx. 0.04µg/g. (The concentration for other pesticides was in the range 0.04 to 0.06µg/g.) There is no significant interference from other constituents and there is good separation.

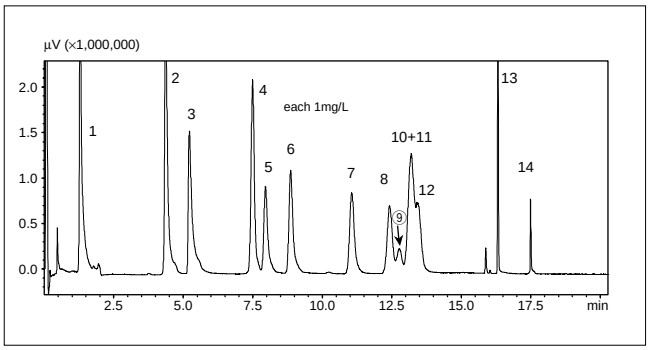


Fig. 1.5.2 Chromatogram for standard mixture of organophosphorus pesticides including phoxim (1mg/L)

Table 1.5.1 Conditions for analysis using notified method

Instrument	: GC-17AAF, FPD-17c, AOC-20i
Column	: CBP-1 10m × 0.53mm I.D. df = 1.0µm
Col.Temp.	: 50°C(1min)-30°C/min-150°C(12min) -30˚C/min-250˚C
Carrier gas	: He, 20mL/min
Detector	: FPD-17c (P Mode), 280ºC
Injection-inlet temperature	: 150ºC
Injection method	: Splitless (1min)
Injection volume	: 1µL

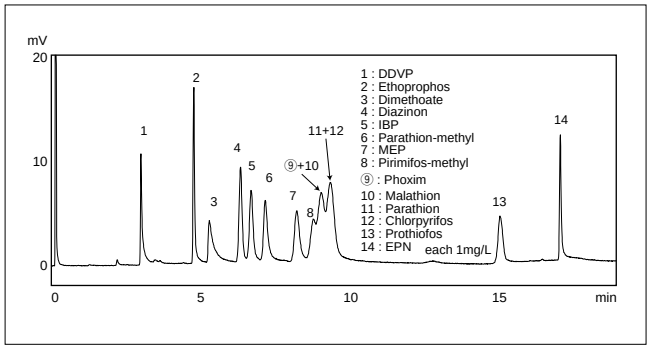


Fig. 1.5.1 Chromatogram for standard mixture of organophosphorus pesticides including phoxim (1mg/L)

Table 1.5.2 Conditions for analysis using short capillary column

Instrument	: GC-2010AF, FPD-2010, AOC-20i
Column	: CBP-1 5m × 0.22mm I.D. df = 0.25µm
Col.Temp.	: 60ºC (1min) - 30ºC/min - 130ºC (12min) - 30ºC/min - 250ºC
Carrier gas	: He, 50kPa (1.8mL/min)
Detector	: FPD-2010 (P Mode) 280ºC
Injection-inlet temperature	: 150ºC
Injection method	: High-pressure splitless (350kPa, 1min)
Injection volume	: 2µL

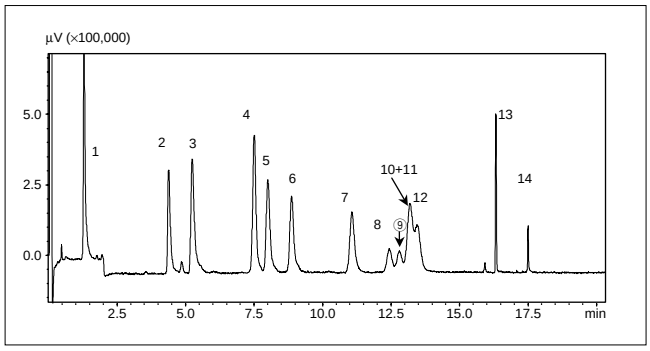


Fig. 1.5.3 Chromatogram for processed soybean solution to which organophosphorus pesticides including phoxim have been added (phoxim: 0.04µg/g; other pesticides: 0.04 to 0.06µg/g)

1.6 Simultaneous Analysis of Pesticides (1) - GC/MS

■Explanation

Residual Pesticides on vegetables and fruits are a matter of concern. There are various kinds of pesticides used, among which approximately 240 are subjected to regulations in Japan. A good way of analyzing these pesticides is simultaneous GC/MS measurement.
Here, an example of a simultaneous analysis of 86 pesticides using GC/MS is shown.

■Analytical Conditions

Instrument : GCMS-QP5000
Column : DB-1 30m × 0.25mmI.D. df=0.25μm
Col.Temp. : 50°C(2min)-20°C/min-130°C
-3°C/min-300°C(7min)
Inj.Temp. : 280°C
I/F Temp. : 280°C
Carrier Gas : He 120kPa(2min)-2kPa/min-250kPa

Table 1.6.1 List of pesticides and molecular weights

	Component	Molecular weight
1	Methamidophos	141
2	Dichlorvos	220
3	Propamocarb	188
4	Acephate	183
5	Isoprocarb	193
6	Fenobucarb	207
7	Ethoprophos	242
8	Chlorproham	213
9	Bendaiocarb	223
10	Dimethipin	210
11	α-BHC	288
12	Dimethoate	229
13	Thiometon	246
14	β-BHC	288
15	γ-BHC	288
16	σ-BHC	288
17	Terbufos	288
18	Diazinon	304
19	Ethiofencarb	225
20	Etrimfos	292
21	Pirimicarb	238
22	Metribuzin	214
23	Bentazone	254
24	Parathion-methyl	263
25	Carbaryl	201
26	Heptachlor	370
27	Fenitrothion	277
28	Methiocarb	225
29	Dichlofluanid	332
30	Esprocarb	265
31	Pirimifos-methyl	305
32	Thiobencarb	257
33	Malathion	330
34	Aldrin	362
35	Fenthion	278
36	Parathion	291
37	Chlorpyrifos	349
38	Diethofencarb	267
39	Captan	299
40	Heptachlor epoxide	386
41	Pendimethalin	281
42	α-Chlorfenvinphos	358
43	Pyrifenox	294

	Component	Molecular weight
44	Chinomethionat	234
45	β-Chlorfenvinphos	358
46	Quinalphos	298
47	Phenthoate	320
48	Triadimenol	295
49	Vamidothion	287
50	Trichlamide	339
51	Methoprene	310
52	Flutolanil	323
53	Dieldrin	378
54	Prothiofos	344
55	Myclobutanil	288
56	p,p'-DDE	316
57	Pretilachlor	311
58	Endrin	378
59	Fensulfothion	308
60	Chlorobenzilate	324
61	p,p'-DDD	318
62	o,p'-DDT	352
63	Mepronil	269
64	Lenacil	234
65	Edifenphos	310
66	Captafol	347
67	p,p'-DDT	352
68	Propiconazole	341
69	EPN	323
70	Dicofol	370
71	Phosalone	367
72	Mefenacet	298
73	Amitraz	293
74	Cyhalothrin	449
75	Bitertanol	337
76	Pyridaben	364
77	Inabenfide	338
78	Permethrin	390
79	Cyfluthrin	363
80	Cypermethrin	415
81	Flucythrinate	451
82	Fenvalerate	419
83	Fluvalinate	502
84	Pyrazoxyfen	437
85	Deltamethrin	503
86	Tralomethrin	661

1.6 Simultaneous Analysis of Pesticides (2) - GC/MS

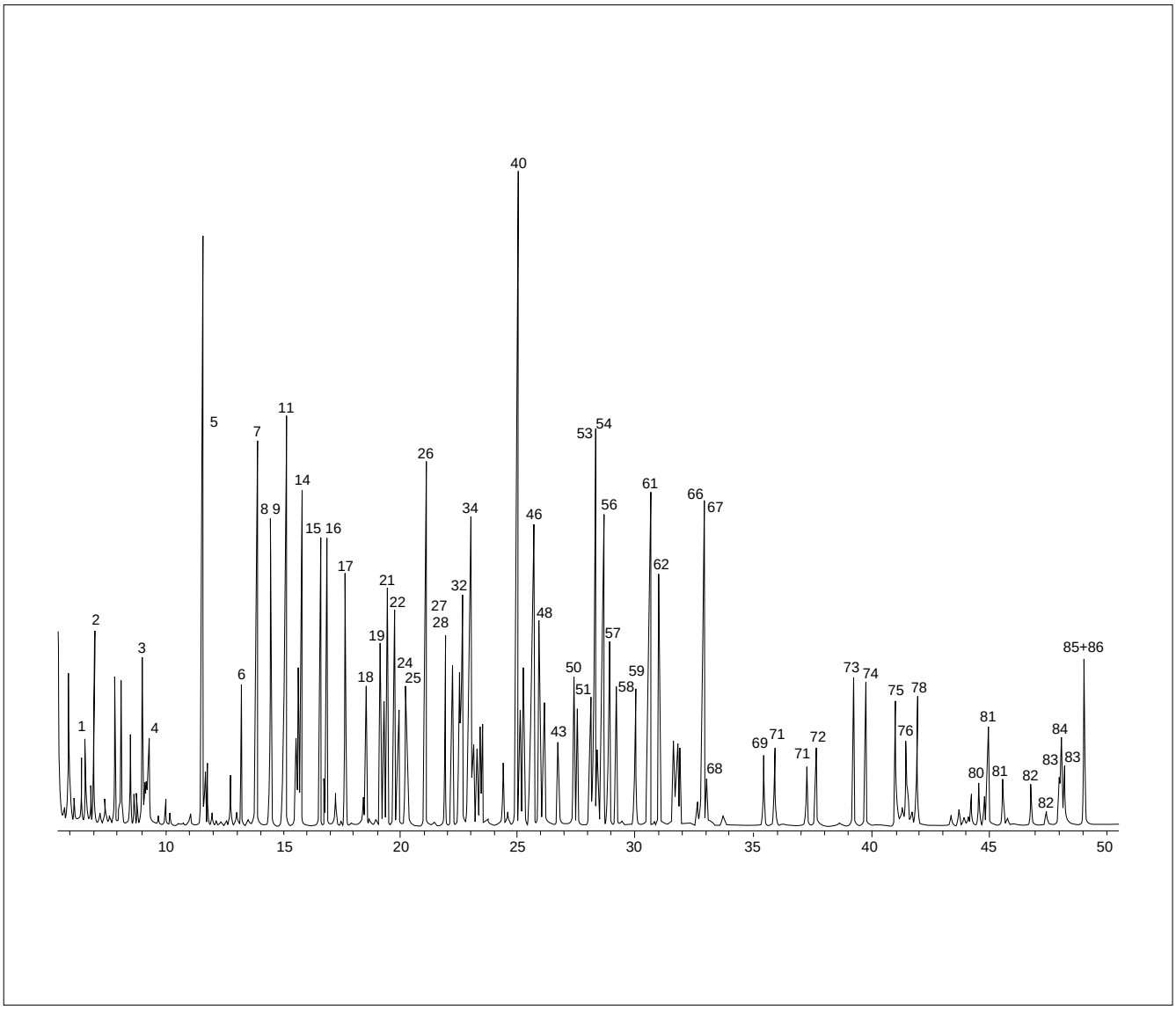


Fig. 1.6.1 Analysis of 86 pesticides using DB-1

1.7 Analysis of Pesticides Using NCI (1) - GC/MS

■Explanation

Trace analysis is required for the measurement of residual pesticides in vegetables and fruits, but it is difficult to extract only pesticides, even after a cleanup pretreatment.
NCI is an effective method for this analysis.
Generally, positive ions are detected in mass spectrometry, but negative-ion analysis may be used depending on the compound. The negative ions of such compounds allow microanalysis with minimal interference from the matrix. Trace amount of pesticides that cannot be detected using the conventional EI method can be detected by this method.

■Analytical Conditions

Instrument	: GCMS-QP5050A
Column	: DB-1 30m × 0.25mmI.D. df=0.25µm
Col.Temp.	: 50°C(2min)-20°C/min-130°C -3°C/min-300°C(7min)
Inj.Temp.	: 280°C
I/F Temp.	: 280°C
Carrier Gas	: He 120kPa(2min)-2kPa/min-250kPa

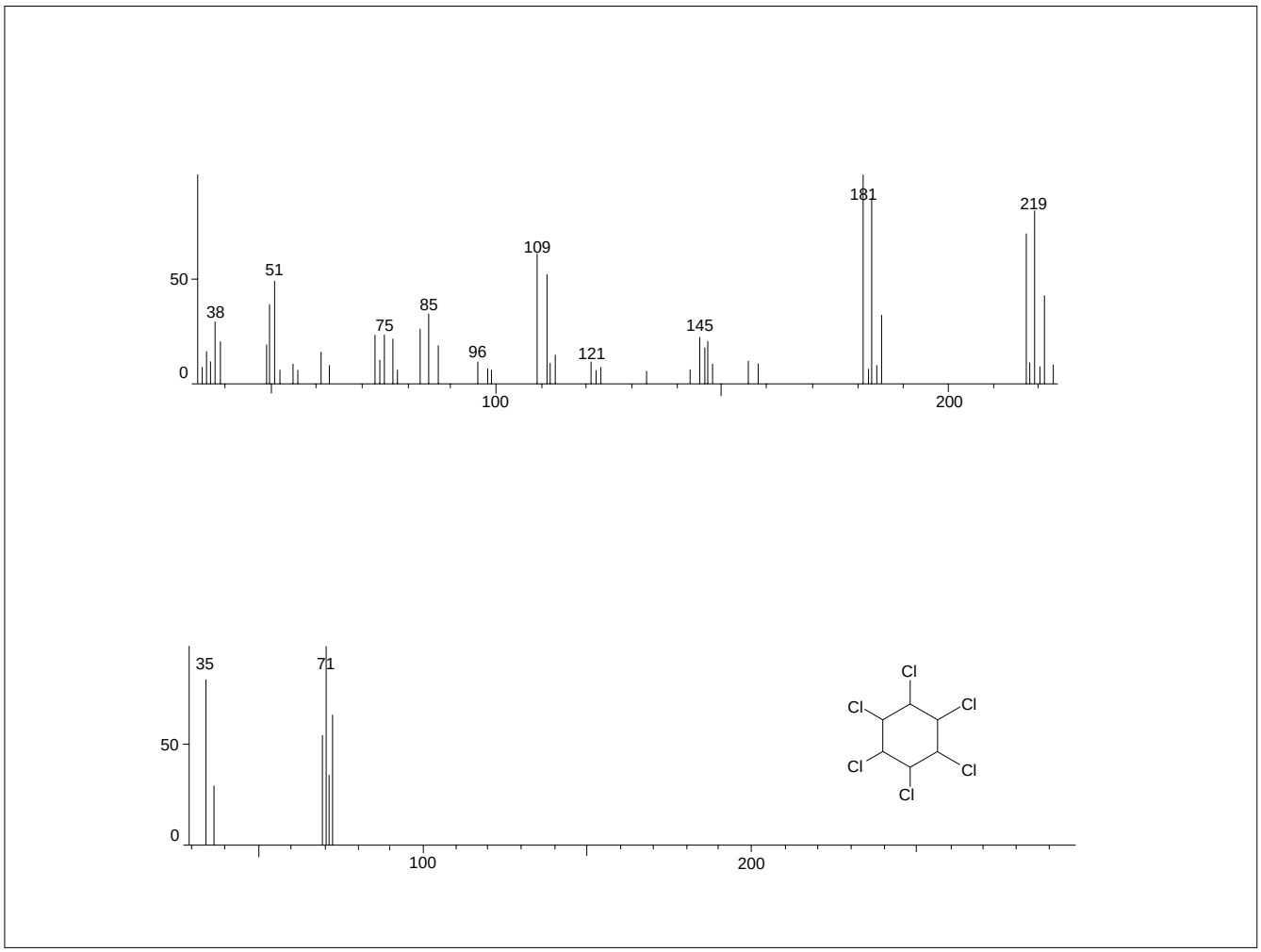


Fig. 1.7.1 α-BHC mass spectrum (upper: EI, lower: NCI)

1.7 Analysis of Pesticides Using NCI (2) - GC/MS

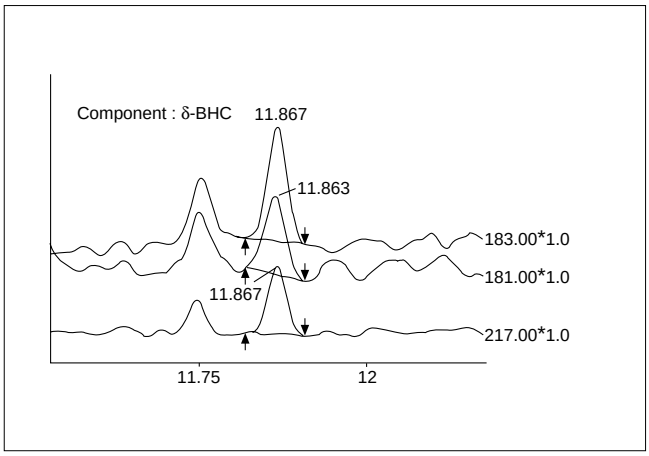


Fig. 1.7.2 SIM chromatogram using EI

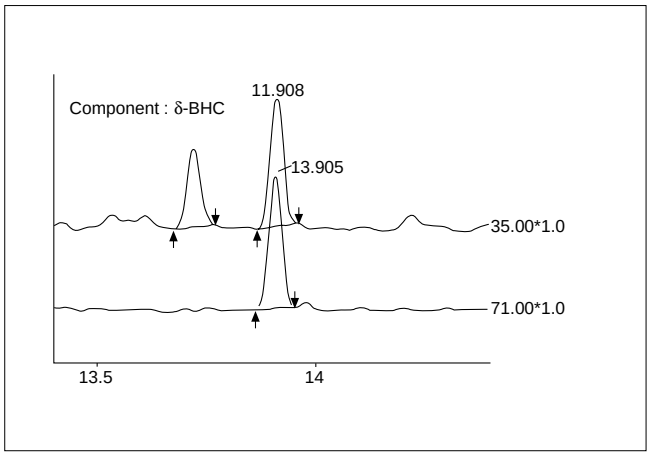


Fig. 1.7.3 SIM chromatogram using NCI

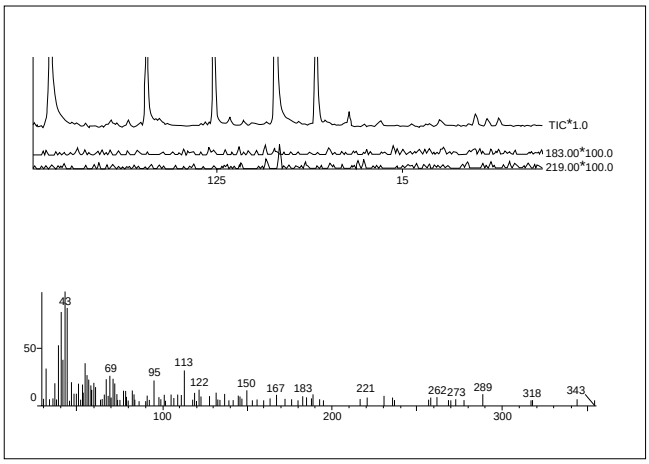


Fig. 1.7.4 MC and mass spectrum using EI

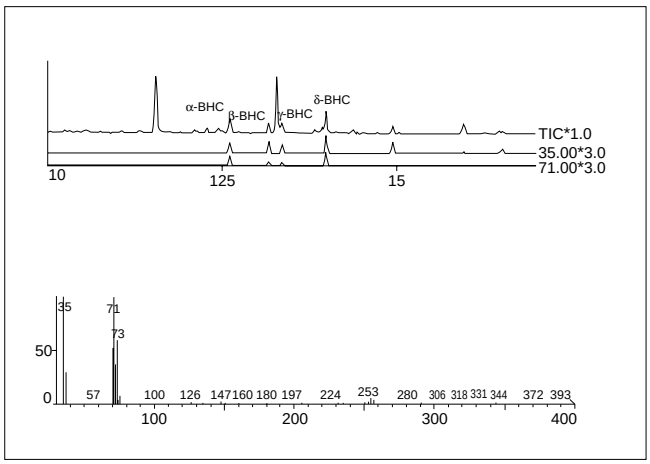


Fig. 1.7.5 MC and mass spectrum using NCI

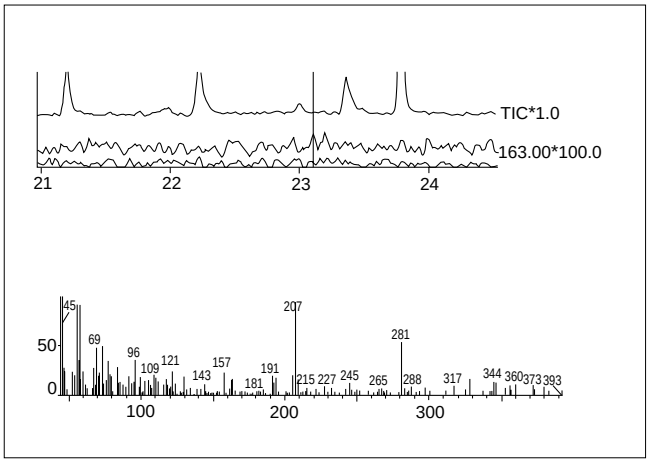


Fig. 1.7.6 MC and mass spectrum using EI

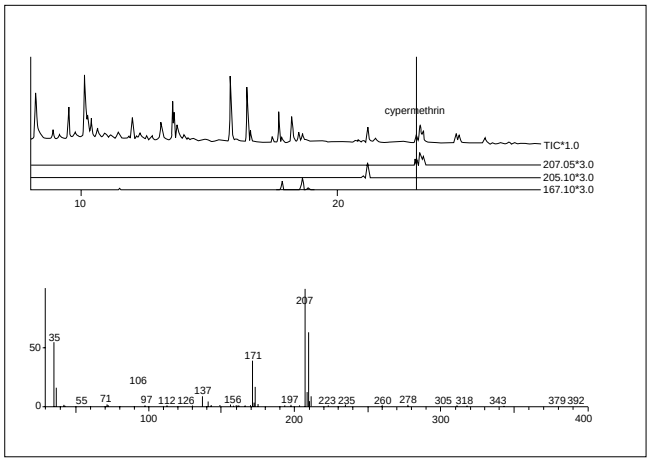


Fig. 1.7.7 MC and mass spectrum using NCI

1.8 Analysis of Residual Pesticides in Food Products Using GC/MS (part 1) (1) - GC/MS

■Explanation

Among pesticides newly regulated in Japan after August 1995, components that can be analyzed by GC/MS or GC were analyze here. The analytical conditions are given in Table 1.8.1 and the analyzed 47 pesticides are listed in Table 1.8.2.

Fig. 1.8.1 shows the TIC chromatogram for the 47 pesticides and Figs. 1.8.2 to 1.8.5 show the SIM chromatograms. The concentration of each substance is 1 ppm.

Table 1.8.1 Analytical Conditions

Instrument	: GCMS-QP5050A
-GC-	
Column	: DB-1 30m × 0.25mm I.D. df=0.25μm
Column temperature	: 50°C(1min)-20°C/min-120°C-5°C/min -300°C(1.5min)
Injection inlet temperature	: 300°C
Interface temperature	: 300°C
Carrier Gas	: He 250kPa(1min)-100kPa(2min) -3kPa/min-220kPa
Injection method	: Splitless (2min)
-MS-	
Scan range	: m/z 35→550
SIM	: 0.2sec

Table 1.8.2 List of Pesticides

Peak NO.	Compound	SIM			Peak NO.	Compound	SIM	
1	DCIP	121.05	77.05	123.05	28	Lenacil	153.15	234.20
2	EPTC	128.20	189.10		29	Thenylchlor	288.25	127.15
3	Butylate	156.20	217.10		30	Acetamiprid	152.15	221.00
4	Cadusafos	159.00	270.25		31	Tebuconazole	250.15	125.10
5	Trifluralin	306.10	264.05		32	Pyributicarb	181.15	165.15
6	Thiometon	125.05	88.10	246.20	33	Bifenthrin	181.20	166.20
7	Nitenpyram	236.05	169.05		34	Tebufenpyrad	333.30	318.25
8	Tefluthrin	197.10	177.10		35	Furametpyr	298.30	157.10
9	Dimethenamid	230.10	154.15		36	Pyriproxyfen	136.20	226.20
10	Tolclophos-methyl	265.05	267.05		37	Cyhalofop-butyl	357.30	256.20
11	Alachrol	188.15	160.20		38	Fenarimol	330.10	139.10
12	Dimethylvinphos(E)	295.05	297.05		39	Pyraclofos	360.05	194.10
13	Pirimifos-methyl	305.20	290.20		40	Acrinathrin	181.15	289.10
14	Dimethylvinphos(Z)	295.05	297.05		41	Pyridaben	147.20	364.10
15	Metolachlor	238.15	162.20		42	Cafenstrole	100.15	188.20
16	Fosthiazate	195.15	283.00		43	Cyfluthrin	163.15	226.00
17	Fosthiazate	195.15	283.00		44	Cyfluthrin	163.15	226.00
18	Isofenphos	213.15	255.10		45	Cyfluthrin	163.15	266.00
19	Paclobutrazol	236.15	125.10		46	Cyfluthrin	163.15	266.00
20	Tricyclazole	189.10	162.10		47	Halfenprox	265.10	183.10
21	Fludioxonil	248.15	182.05		48	Pyrimidifen	184.15	377.00
22	Butamifos	286.20	200.15		49	Fenvalerate	125.10	418.95
23	Myclobutanil	179.10	288.15		50	Fenvalerate	125.10	418.95
24	Difenzoquat	234.20	189.10		51	Difenoconazole	323.15	265.10
25	Flusilazole	233.15	315.15		52	Difenoconazole	323.15	265.10
26	Cyproconazole	222.10	139.10		53	Imibenconazole	375.20	125.05
27	Chlorphenapyr	59.10	247.00					

1.8 Analysis of Residual Pesticides in Food Products Using GC/MS (part 1) (2) - GC/MS

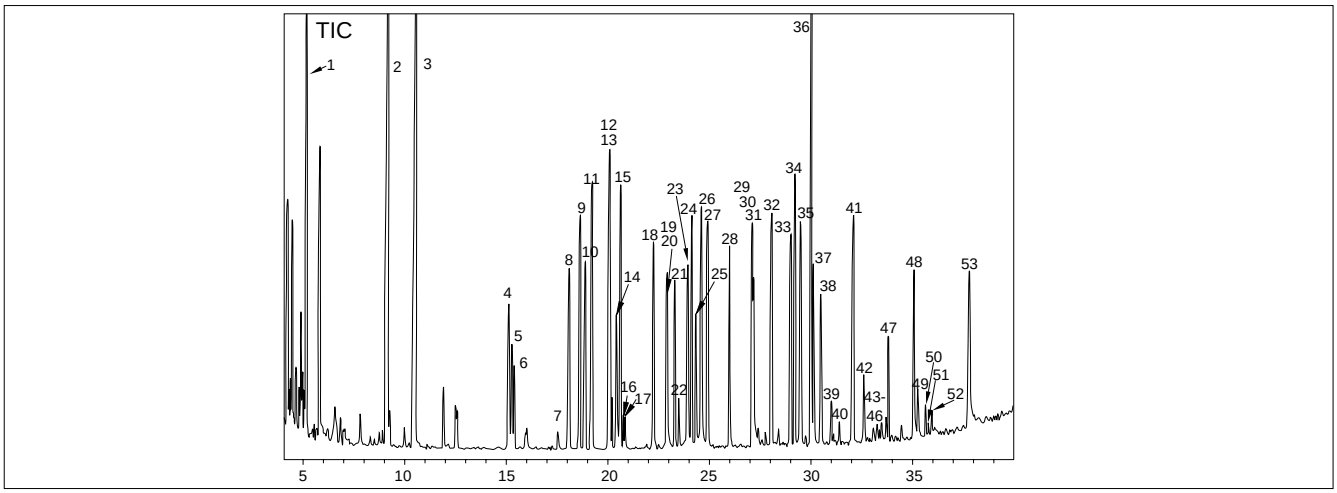


Fig. 1.8.1 TIC Chromatogram of Pesticides

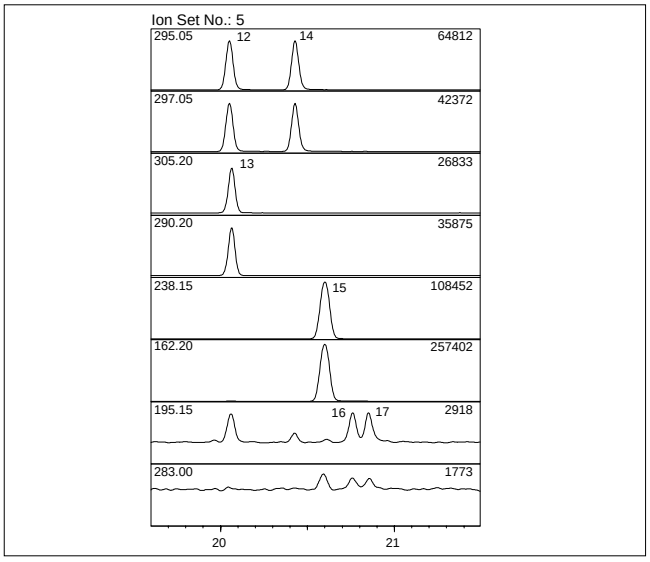


Fig. 1.8.2 SIM Chromatogram of Pesticides (ion set: 5)

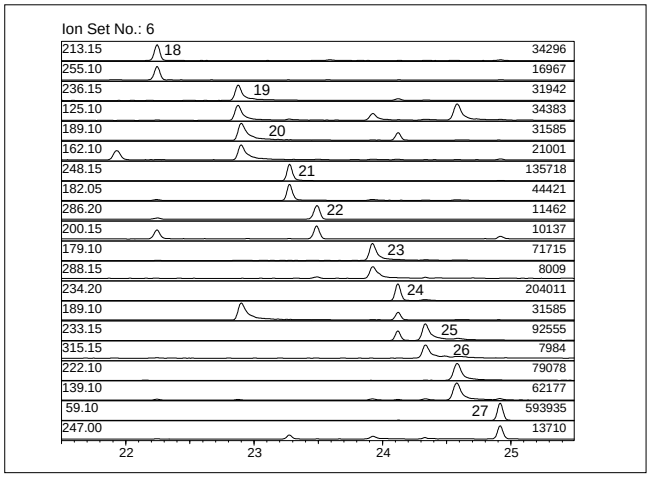


Fig. 1.8.3 SIM Chromatogram of Pesticides (ion set: 6)

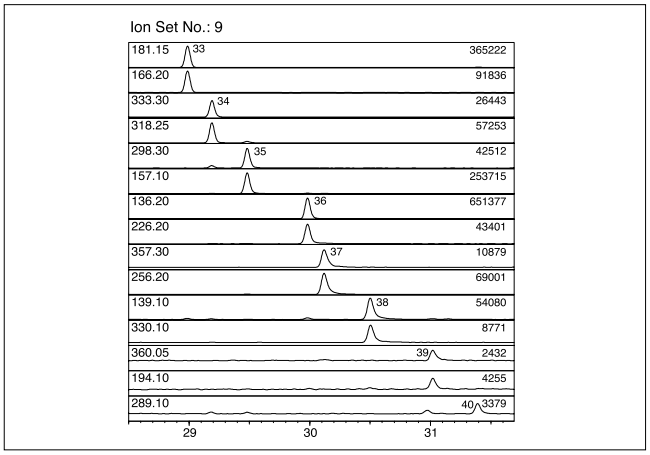


Fig. 1.8.4 SIM Chromatogram of Pesticides (ion set: 9)

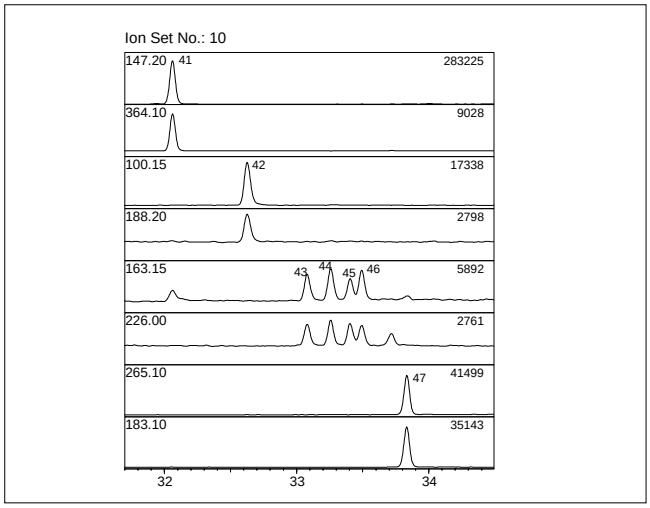


Fig. 1.8.5 SIM Chromatogram of Pesticides (ion set: 10)

1.9 Analysis of Residual Pesticides in Food Products Using GC/MS (part 2) (1) - GC/MS

■Explanation

Among pesticides newly regulated in Japan after August 1995, components that can be analyzed by GC/MS NCI (Negative Chemical Ionization) method were introduced here.
This article introduces examples of analyzing these pesticides by the NCI (negative chemical ionization) method. The NCI method allows detection with higher sensitivity than the EI method for some compounds. Though the NCI method is especially effective for compounds containing chlorine, this article also shows examples of compounds not containing chlorine.

The EI and NCI mass spectra, as well as the SIM chromatograms at 10ppb for isofenphos, pyributicarb and fenvalerate are shown in Figs 1.9.1 to 1.9.3 respectively. Isofenphos and pyributicarb are pesticides that do not contain chlorine.
Detection of 10ppb fenvalerate is difficult with the EI method but the NCI method allows high sensitivity detection. Compounds not containing chlorine such as isofenphos and pyributicarb can also be detectable by the NCI method.

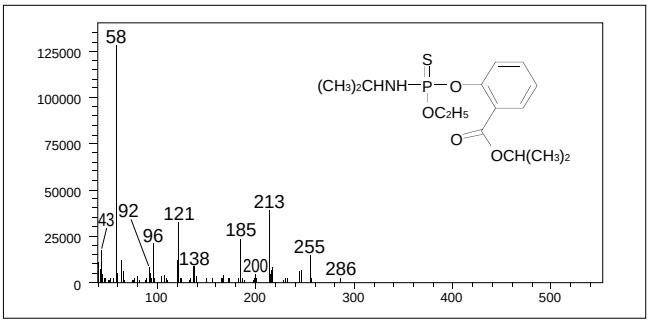


Fig. 1.9.1 EI mass spectrum of isofenphos

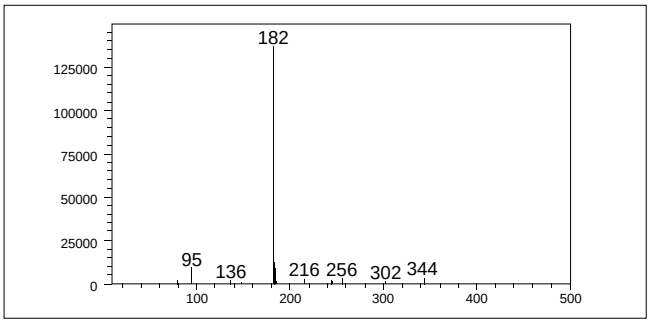


Fig. 1.9.2 NCI mass spectrum of isofenphos

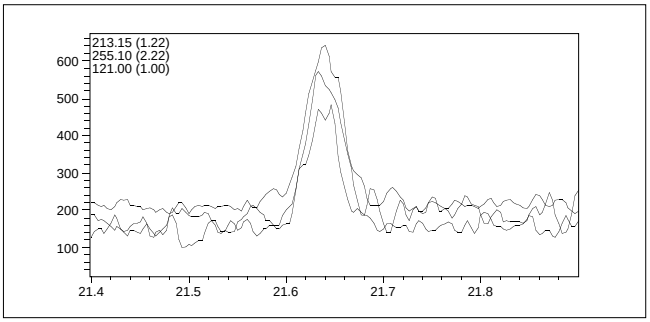


Fig. 1.9.3 SIM chromatogram of isofenphos (EI method, 10ppb)

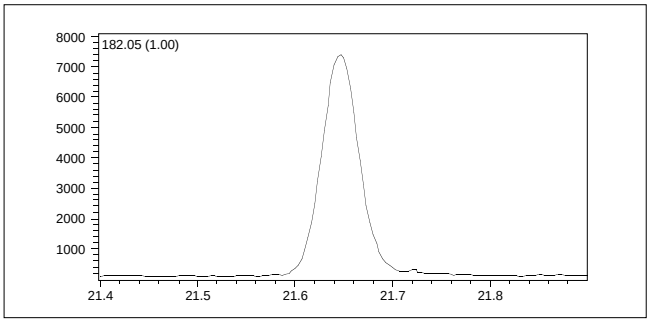


Fig. 1.9.4 SIM chromatogram of isofenphos (NCI method, 10ppb)

1.9 Analysis of Residual Pesticides in Food Products Using GC/MS (part 2) (2) - GC/MS

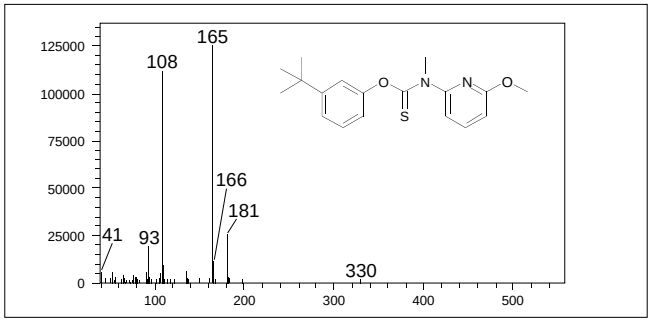


Fig. 1.9.5 EI mass spectrum of Pyributicarb

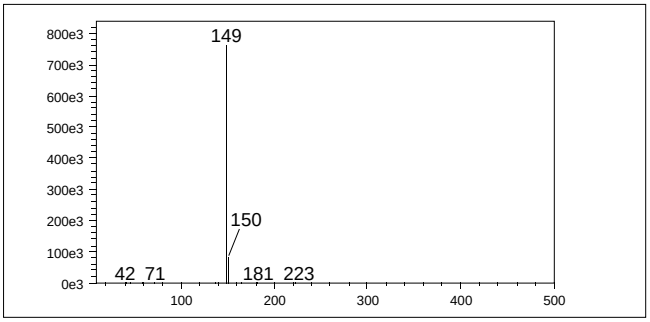


Fig. 1.9.6 NCI mass spectrum of Pyributicarb

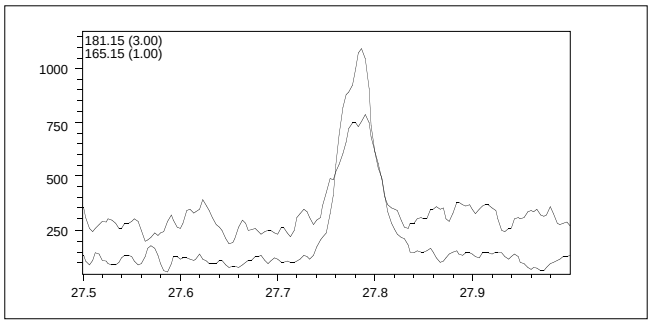


Fig. 1.9.7 SIM chromatogram of Pyributicarb (EI method, 10ppb)

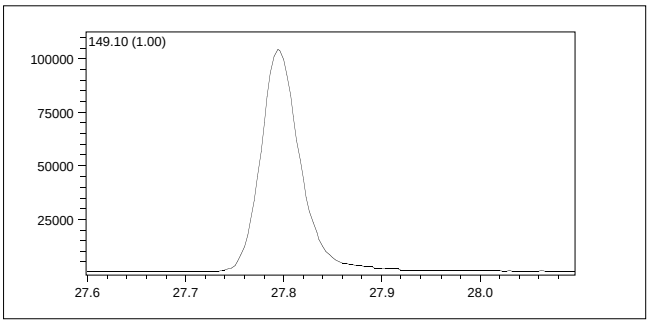


Fig. 1.9.8 SIM chromatogram of Pyributicarb (NCI method, 10ppb)

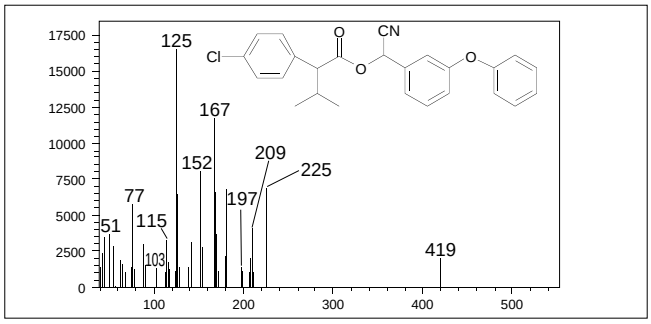


Fig. 1.9.9 EI mass spectrum of Fenvalerate

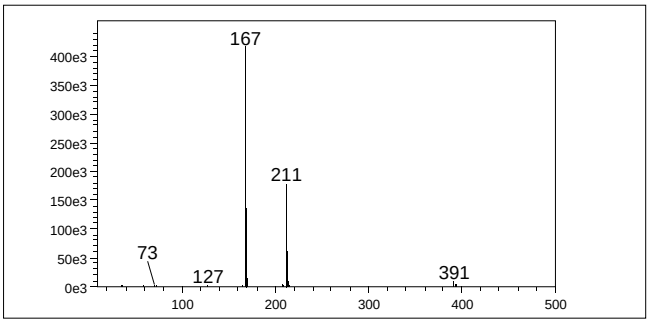


Fig. 1.9.10 NCI mass spectrum of Fenvalerate

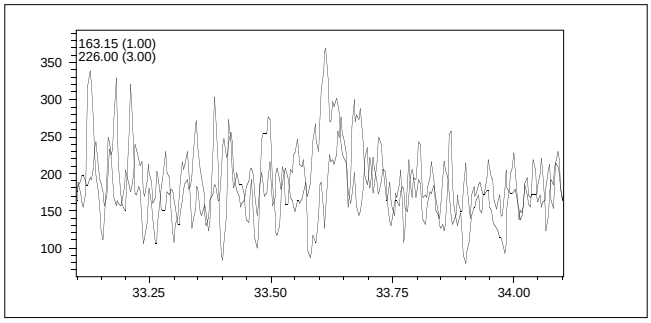


Fig. 1.9.11 SIM chromatogram of Fenvalerate (EI method, 10ppb)

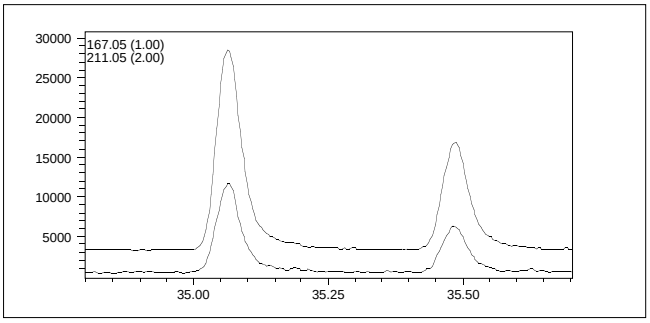


Fig. 1.9.12 SIM chromatogram of Fenvalerate (NCI method, 10ppb)

Table 1.9.1 Analytical Conditions

GCMS	:	Shimadzu GCMS-QP5050A			
-GC-			-MS-		
Column	:	DB-5 (30m × 0.25mm I.D. df=0.25μm)	Interface Temp.	:	300˚C
Column Temp.	:	50˚C(1min)-20˚C/min-100˚C-5˚C/min-300˚C(1.5min)	Scan Range	:	EI m/z 35~550 NCI m/z 10~550
Injector Temp.	:	300˚C	Ionization Method	:	NCI (iso-Butane)
Carrier Gas	:	He 100kPa(2min)-3kPa/min-220kPa(3min) Splitless(2min)			

1.10 Analysis of Pesticide Residue in Vegetable Juice Using GC/MS (1) - GC/MS

■Explanation

After extracting Vegetable juice with n-hexane (concentration factor of 10), standard pesticide products were added to concentrations of 20ng/mL and then analyzed with GC/MS. Library searches and quantitative analysis were performed on the three representative components for which the elution positions are indicated with arrows in Fig. 1.10.2. Clean-up was not performed for the sample.

■Analytical Conditions

Instrument	: GCMS-QP2010
Column	: ZB1 30m × 0.32mm I.D., df = 0.25μm)
Column temp.	: 70˚C(1min)-20˚C/min-120˚C/min-10˚C/min-270˚C(4min)
Inj. Temp.	: 270˚C
I/F Temp.	: 250˚C
Ion source temperature	: 200˚C

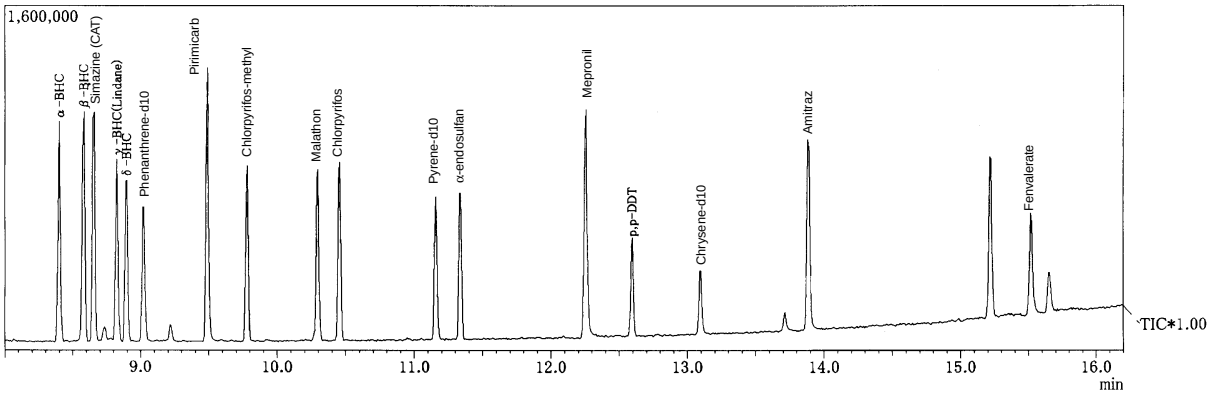


Fig. 1.10.1 TIC chromatogram of standard pesticides (1ppm each)

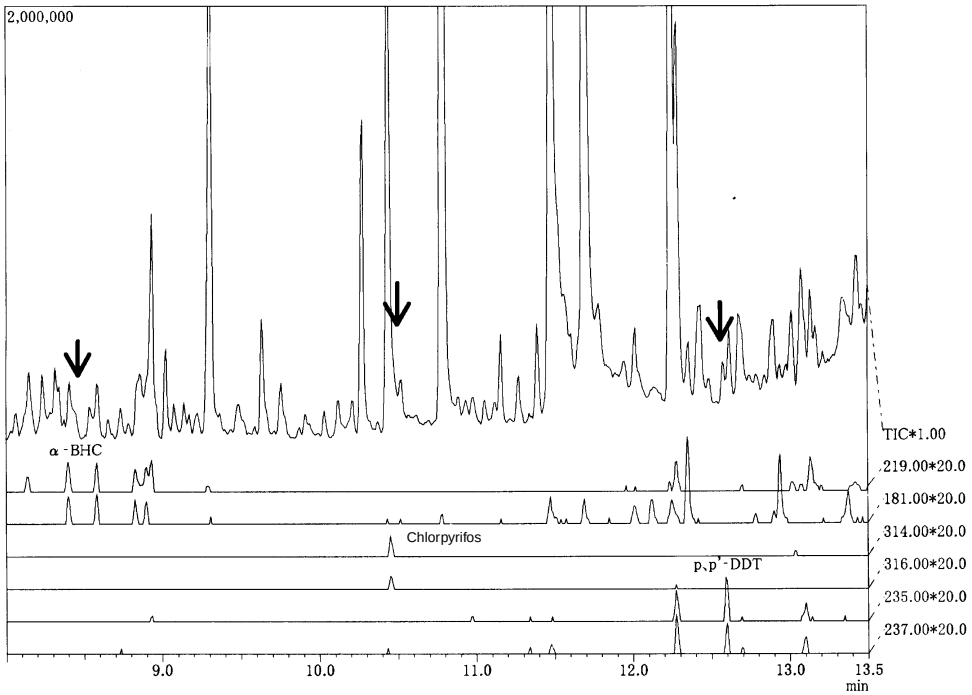


Fig. 1.10.2 TIC chromatogram (enlarged) and mass chromatogram of Vegetable juice

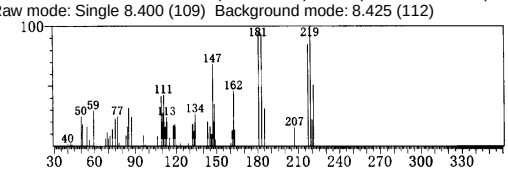
1.10 Analysis of Pesticide Residue in Vegetable Juice Using GC/MS (2) - GC/MS

■Results of Library Searches and Quantitative Analysis

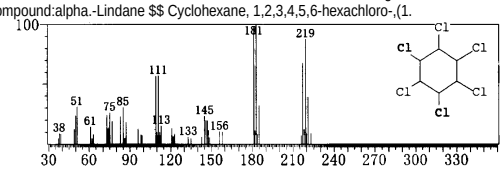
α-BHC

Library search results

Target
Line #: 1 Retention time: 8.400 (scan #: 109) Mass peak #: 56 Base peak
Raw mode: Single 8.400 (109) Background mode: 8.425 (112)

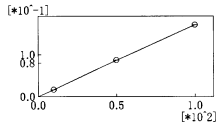


Hit #: 1 Entry #: 67749 Library: NIST 107.LIB
SI: 74 Molecular formula: C6H6Cl6 CAS: 319-84-6 Molecular weight: 288 Retention index: 0
Compound:alpha.-Lindane $$ Cyclohexane, 1,2,3,4,5,6-hexachloro-,(1.



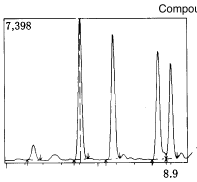
Compound: a-BHC

m/z: 181.00 Calibration curve: Quadratic Origin: Through origin Internal standard method
f(x)=-0.000000*x^2+0.001749*x+0.000000
Correlation coefficient (R) = 0.999982 Contribution (R'2): 1.000000



#	Concentration (ppb)	Area ratio
1	10.000	0.02
2	50.000	0.09
3	100.000	0.17

Compound: α-BHC

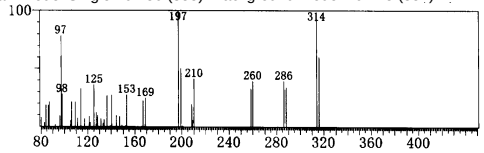


ID #: 1 m/z: 181.00
Type: Target
Retention time: 8.405
Area: 9821
Concentration: 26.201ppb

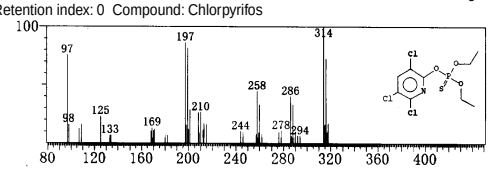
Chlorpyrifos

Library search results

Target
Line #: 4 Retention time: 10.450 (scan #: 355) Mass peak #: 46 Base peak
Raw mode: Single 10.450 (355) Background mode: 10.425 (352)

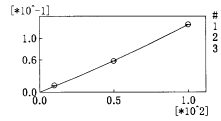


Hit #: 1 Entry #: 19366 Library: NIST 21.LIB
SI: 66 Molecular formula: C9H11Cl3NO3PS CAS: 2921-88-2 Molecular weight: 349
Retention index: 0 Compound: Chlorpyrifos



Compound: Chlorpyrifos

m/z: 314.00 Calibration curve: Quadratic Origin: Through origin Internal standard method
f(x)=-0.000002*x^2+0.001070*x+0.000000
Correlation coefficient (R) = 0.998496 Contribution (R'2): 0.999639

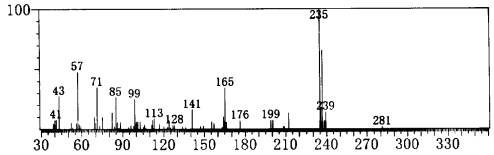


#	Concentration (ppb)	Area ratio
1	10.000	0.01
2	50.000	0.06
3	100.000	0.12

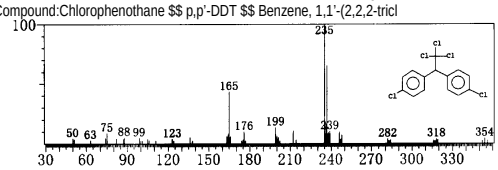
p,p'-DDT

Library search results

Target
Line #: 5 Retention time: 12.592 (scan #: 612) Mass peak #: 69 Base peak
Raw mode: Average 12.592-12.600 (612-613) Background mode: Average 1

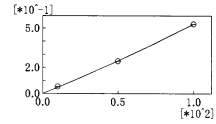


Hit #: 1 Entry #: 84517 Library: NIST 107.LIB
SI: 62 Molecular formula: C14H9Cl5 CAS: 50-29-3 Molecular weight: 352 Retention index: 0
Compound:Chlorophenothane $$ p,p'-DDT $$ Benzene, 1,1'-(2,2,2-tricl



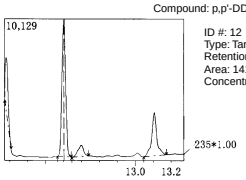
Compound: p,p'-DDT

m/z: 235.00 Calibration curve: Quadratic Origin: Through origin Internal standard method
f(x)=-0.000006*x^2+0.004688*x+0.000000
Correlation coefficient (R) = 0.998956 Contribution (R'2): 0.999738



#	Concentration (ppb)	Area ratio
1	10.000	0.05
2	50.000	0.25
3	100.000	0.53

Compound: p,p'-DDT



ID #: 12 m/z: 235.00
Type: Target
Retention time: 12.602
Area: 14105
Concentration: 30.207ppb

1.11 Analysis of Residual Organophosphorus Pesticides in Agricultural Products (1) - GC/MS

Analysis Complying with Standards for Food Products and Additives Specified in the Food Sanitation Law (Ministry of Health, Labour and Welfare, Notification No. 370)

■Explanation

In the example presented here, organophosphorus pesticides are analyzed using GC/MS. According to the notified method, if a concentration exceeding the specified limit is measured using GC, a confirmation test must be performed using GC/MS. Here, pretreatment was performed in accordance with the notified method and measurements were obtained using GC/MS. Fig. 1.11.1 shows a scan-measurement total ion chromatogram (TIC) for a standard mixture of organophosphorus pesticides in a 1mg/L solution. Fig. 1.11.2 shows an SIM chromatogram for a standard mixture of organophosphorus pesticides in a 0.1mg/L solution, divided into parts corresponding to different constituents. The top row shows the ions used for quantitative analysis and the lower rows show the ions used for confirmation. Fig. 1.11.3 shows the calibration curves (0.1 to 1mg/L) for diazinon, chlorpyrifos, and EPN. The standard solution of the organophosphorus mixture was added to soybean and analysis was performed. Fig. 1.11.4 shows the SIM chromatogram for a soybean sample to which the mixture was added so that the concentration for each constituent was 0.1µg/g. The quantitative-analysis ions for each sample constituent were measured without being influenced by unwanted constituents.

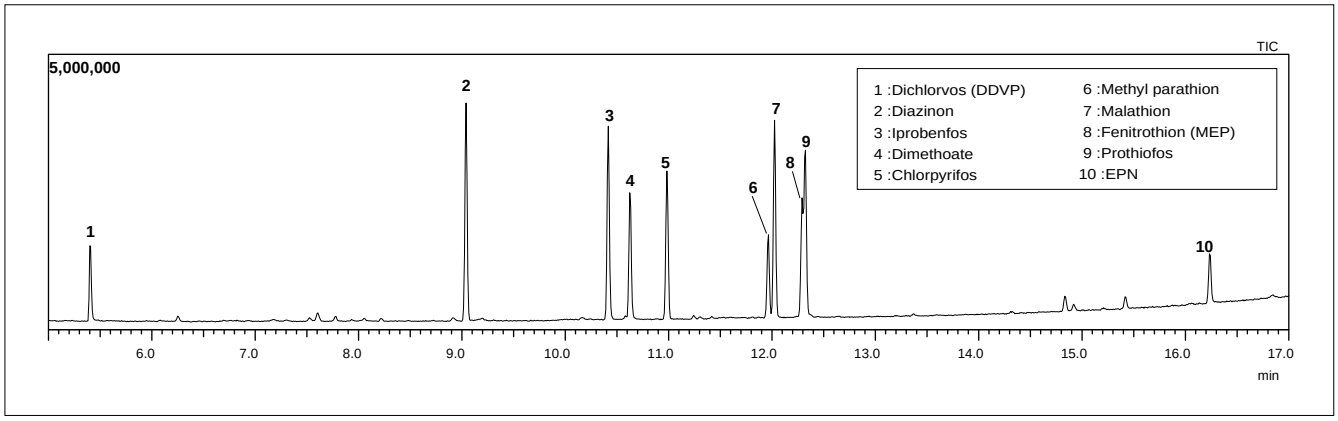


Fig. 1.11.1 Scan-measurement TIC for standard solution of organophosphorus mixture (1mg/L)

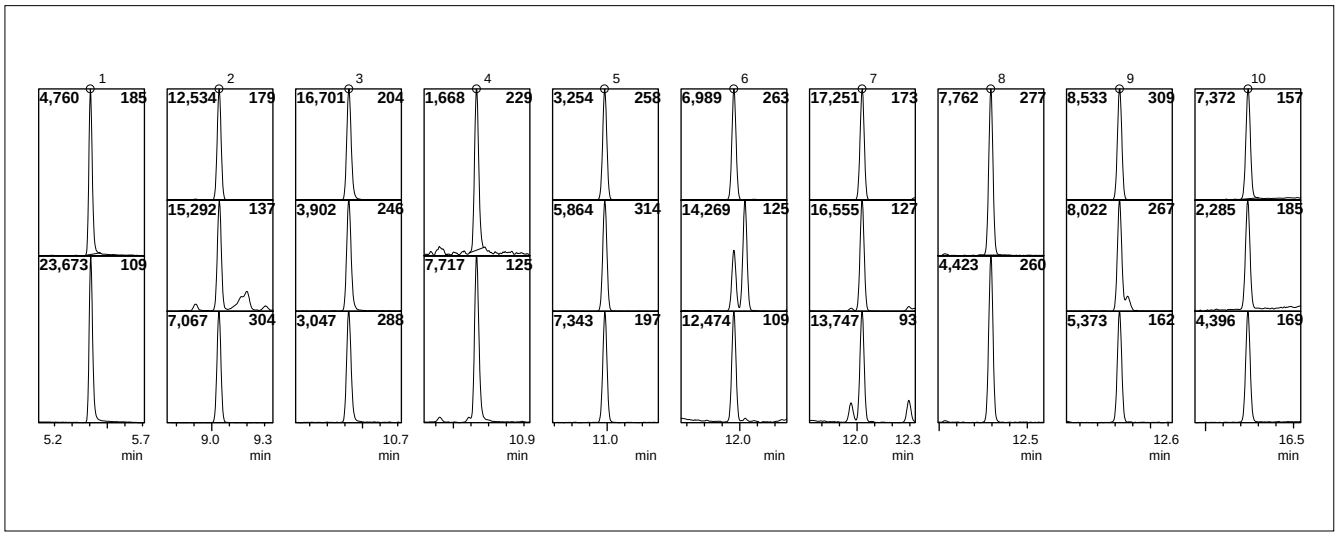


Fig. 1.11.2 SIM chromatogram for standard solution of organophosphorus mixture (0.1mg/L)

1.11 Analysis of Residual Organophosphorus Pesticides in Agricultural Products (2) - GC/MS

Analysis Complying with Standards for Food Products and Additives Specified in the Food Sanitation Law (Ministry of Health, Labour and Welfare, Notification No. 370)

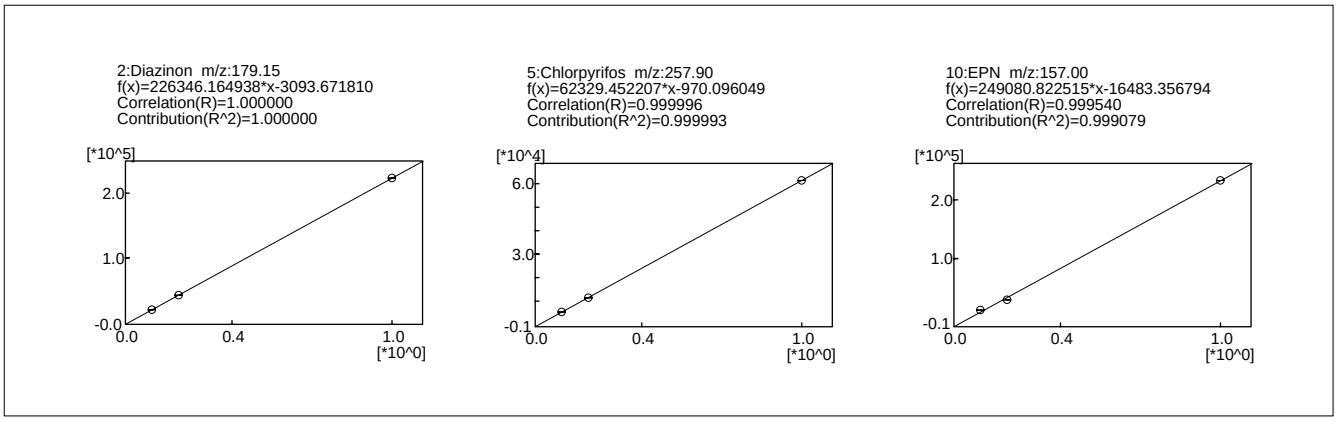


Fig. 1.11.3 SIM calibration curves for diazinon, chlorpyrifos, and EPN (0.1 to 1 mg/L)

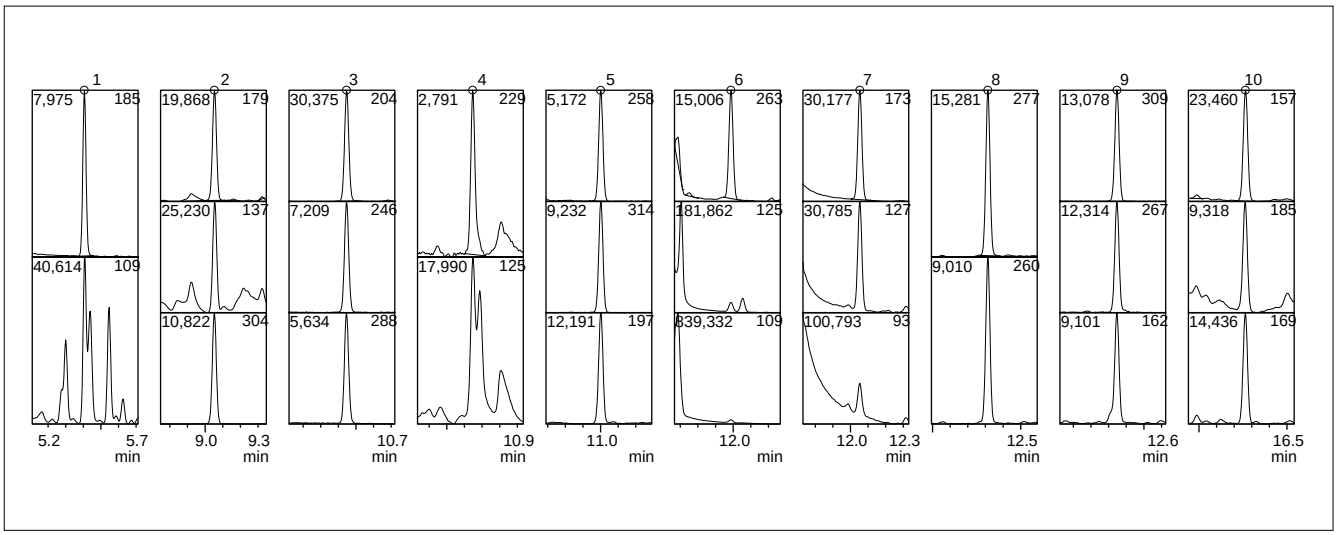


Fig. 1.11.4 SIM chromatogram for soybean to which 0.1µg/gconcentration standard pesticide solution was added

Table 1.11.1 Analytical conditions

Instrument	:	GCMS-QP2010	-MS-	
-GC-			Interface temperature	: 280ºC
Column	:	Rtx-200 30m × 0.32mm I.D. df = 1.5µm	Ion-source temperature	: 200ºC
Column Temp.	:	70ºC (1min)-25ºC/min-125ºC-10ºC/min-280ºC (30min)	Ionization method	: EI
Carrier Gas	:	He, 55kPa (57.8cm/sec, constant line-velocity mode)	Scan range	: 40 to 50
High-pressure injection	:	120kPa	Scan interval	: 0.5sec
Injection-inlet temperature	:	260ºC	SIM interval	: 0.2sec
Injection method	:	Splitless (1min)		
Injection volume	:	1µL		

1.12 Analysis of Residual Pesticides in Foods Using Online GPC-GC/MS (Prep-Q) (1) - GC/MS

■Explanation

In recent years, there has been an increase in regulations regarding residual pesticides in food products and more agricultural products are subject to such regulations. In response to this trend, there has been an increased demand for automating and speeding up the pretreatment procedures for the analysis of residual pesticides. The Japnese Ministry of Health, Labour and Welfare has issued a notice about the rapid method for analyzing residual pesticides. This method employs GPC clean-up for part of pretreatment in order to simultaneously analyze multiple pesticide components (1997 Chemical Hygiene No. 43, 44 and 45).

■Equipment Overview

Samples extracted from food contain large quantities of oils and pigments that will interfere with pesticide analysis. The GPC column separates the fat and pigment substances from the pesticides in the extracted samples in accordance with their molecular size. By switching the valve, fat and pigment substances which elute more quickly are discharged and the target pesticides are taken into the trapping loop. The pesticides trapped in the loop are injected into the GC, separated in the GC column and then detected in the MS section.

■Analytical Conditions

Systems : Prep-Q
GPC : LC-VP Series
GC/MS : GCMS-QP5050A
GPC Column : CLNpak EV-200
(Shodex 150mmL. × 2mm I.D.)
GC Column : J&W
uncoated : deactivated silica tubing
(5m × 0.53mm I.D.)
pre-column : DB-5
(5m × 0.25mm I.D. df=0.25µm)
analysis : DB-5
(30m × 0.25mm I.D. df=0.25µm)

Table 1.12.1

-GPC-	
Mobile phase: Acetone	: Acetone : cyclohexane (3:7)
Flow rate	: 0.1mL/min
Injection volume	: 20µL
Fraction	: 200µL
-GC-	
PTV	: 120°C(0.5min)-80°C/min-280°C(27.5min)
Column temperature	: 82°C(1min)-8°C/min-280°C(4.25min)
Carrier Gas	: He 120kPa
-MS-	
Interface temperature	: 280°C
Scan range	: m/z 86~356
Interval	: 0.5sec

In order to further improve the rapid analysis method, Shimadzu has developed a system that connects the GC/MS and GPC clean-up systems online. By completely automating the GPC and GC/MS processes, the Online GPC-GC/MS (Prep-Q) system realizes simpler and quicker analysis of residual pesticides. Prep-Q was developed under the proposal and directives of the Osaka Prefectural Public Health Laboratory. [1) 2)]

This section shows an analysis example where pesticides were added to an actual sample (potato) and analyzed by Prep-Q.

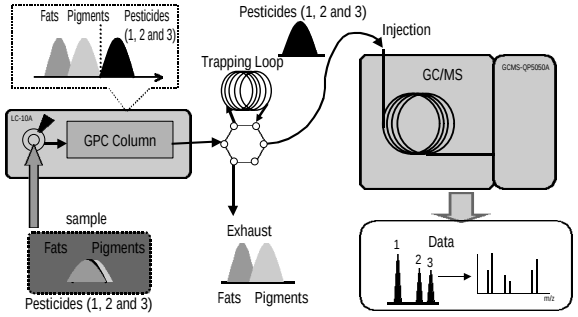


Fig. 1.12.1 Outline of Prep-Q

■Sample Extraction and Pretreatment

Sample pretreatment is performed in accordance with the rapid analysis method for residual pesticides.

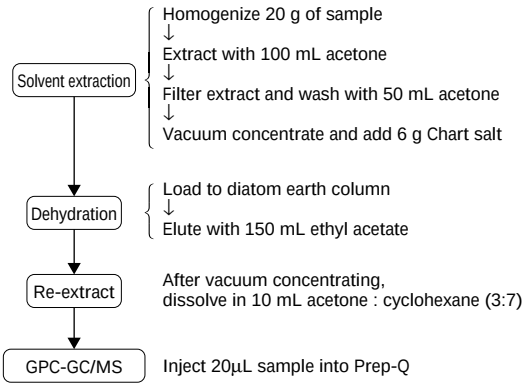


Fig. 1.12.2 Flow of Pretreatment

1.12 Analysis of Residual Pesticides in Foods Using Online GPC-GC/MS (Prep-Q) (2) - GC/MS

■Example of Actual Sample Analysis

In this case, a standard solution of pesticides was added to potato extract and the mixture was analyzed with Prep-Q. The mass chromatograms and mass spectra for four pesticide substances (fenobucarb, BHC, diazinon and permethrin) are provided as an example in Fig. 1.12.3.

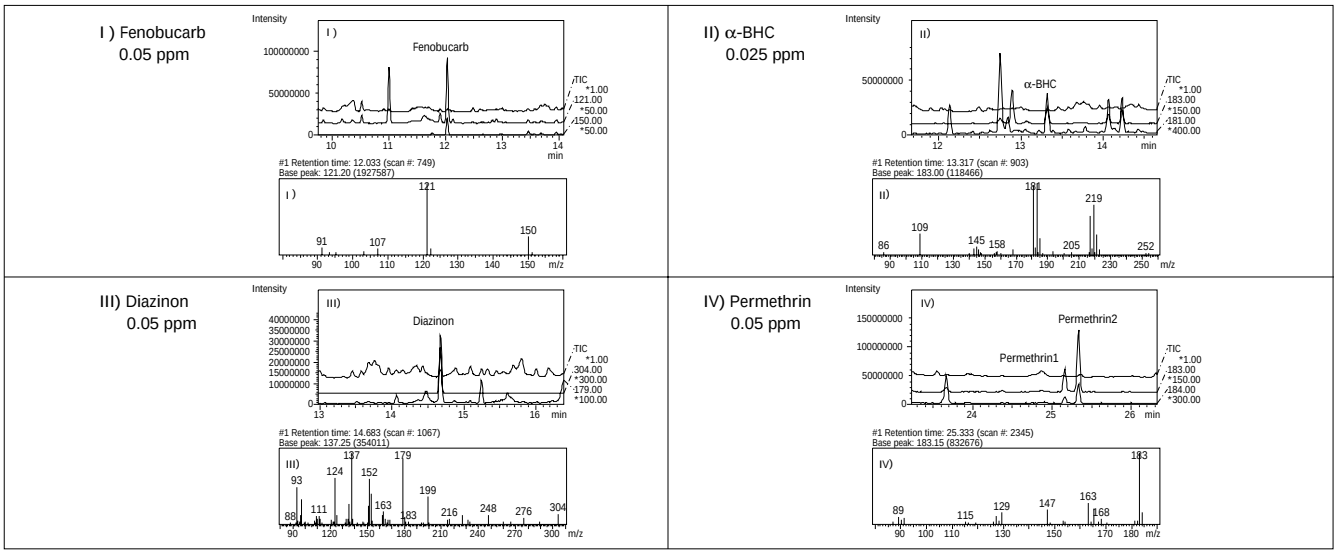


Fig. 1.12.3 Mass Chromatograms and Mass Spectra for Pesticides in Potato Extract

■Comparison to Rapid Analysis Method

Prep-Q employs a small GPC column in the clean-up GPC section in order to reduce the time necessary for the clean-up procedure. In addition, by injecting a large quantity of sample into the GC, the concentration process of the rapid analysis method can be eliminated. As a result, the analysis time per sample was reduced to about one half compared to the conventional rapid analysis method. The amount of solvent used for clean-up GPC was also reduced from 200 mL to 1 mL per sample. Prep-Q enables environmentally friendly and economical analysis of residual pesticides.

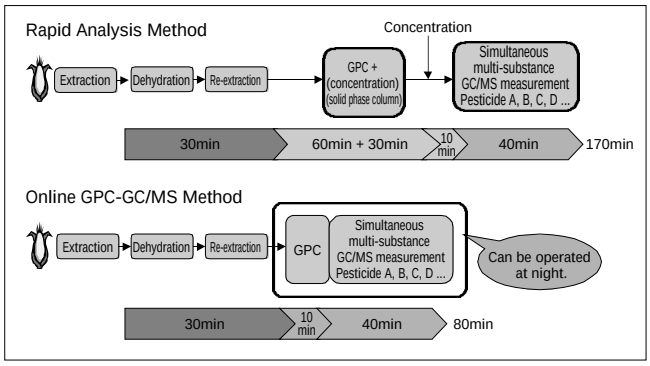


Fig. 1.12.4 Comparison of Analysis Times for Rapid Analysis and On-line GPC-GC/MS Methods

■Conclusion

The Prep-Q system, developed specifically for analyzing residual pesticides in food products, fully automates all procedures from pretreatment and reduces analysis time and solvent consumption. Therefore, residual pesticide analysis can be accomplished more simply and quickly than the conventional rapid analysis method. In addition, since the system is automated, improvements in analytical accuracy and ease of validation (for both equipment and method) can be expected, enabling even more reliable analysis.

■References

1) Study for making the GPC-GC/MS process of analyzing residual pesticides in foods online - large volume injections to GC
Osaka Prefectural Public Health Laboratory: Mikiya Kitagawa, Shinjiro Hori, et al. Food Hygienic Society of Japan, 73rd Technical Symposium
2) Analysis of Residual Pesticides in Foods Using Online GPC-GC/MS
Osaka Prefectural Public Health Laboratory: Mikiya Kitagawa, Shinjiro Hori, et al. Food Hygienic Society of Japan, 77th Technical Symposium

1.13 Analysis of Regulated Pesticides in Foods (1) - LC

■Explanation

The regulations on pesticide residue in foods based on the Food Sanitation Law have undergone many revisions since October 1992 and, as of January 2005, regulated values were specified for 244 of pesticides. The analysis of some standard pesticides for which HPLC is used is described here as an example.

■Analysis of Fenpyroximate

The fenpyroximate content can be obtained from the sum of fenpyroximate-E and fenpyroximate-Z.

■Analytical Conditions

Column : Shim-pack VP-ODS (250mmL. × 4.6mm I.D.)
Mobile phase : Water/Acetonitrile = 1/4 (v/v)
Flow rate : 1.0mL/min
Temperature : 40˚C
Detection : SPD-10AV*VP* 254nm

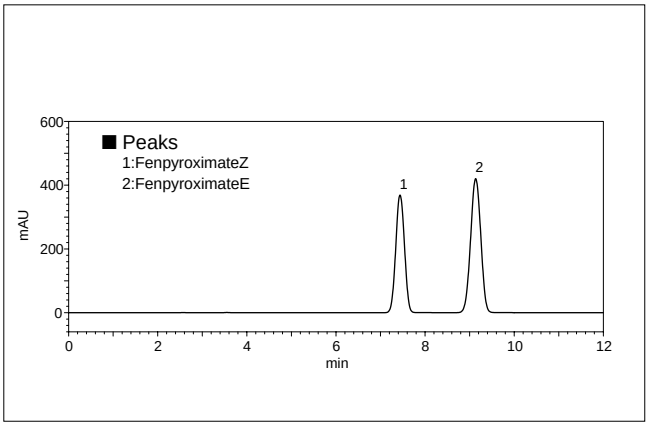


Fig. 1.13.1 Chromatogram of fenpyroximate

■Analysis of Cyromazine

Because cyromazine has a high polarity, there is insufficient retaining power with an ODS column and so an aminopropyl column is used.

■Analytical Conditions

Column : Shodex Asahipak NH2P-50 4E (250mmL. × 4.6mm I.D.)
Mobile phase : Water/Acetonitrile = 7/93 (v/v)
Flow rate : 0.8mL/min
Temperature : 40˚C
Detection : SPD-10AV*VP* 215nm

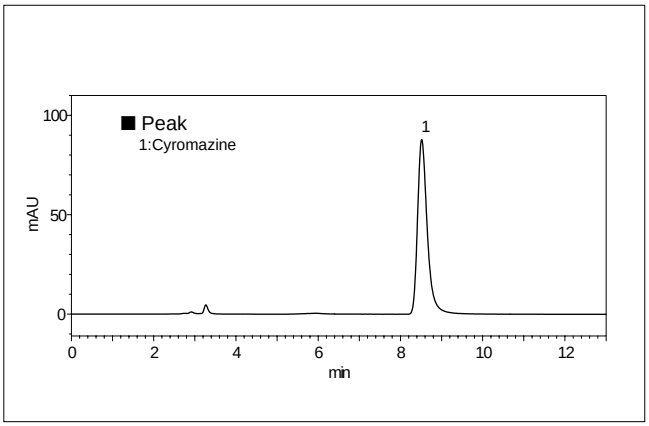


Fig. 1.13.2 Chromatogram of cyromazine

1.13 Analysis of Regulated Pesticides in Foods (2) - LC

■Analysis of Nitenpyram

In the nitenpyram testing method, the nitenpyram content is obtained by analyzing nitenpyram with HPLC and analyzing its CPF metabolite with GC.

■Analytical Conditions

Column : Shim-pack VP-ODS (150mmL. × 4.6mm I.D.)
Mobile phase : 50mM KH_2PO_4/Methanol = 17/3 (v/v)
Flow rate : 0.8mL/min
Temperature : 40˚C
Detection : SPD-10AV*VP* 270nm

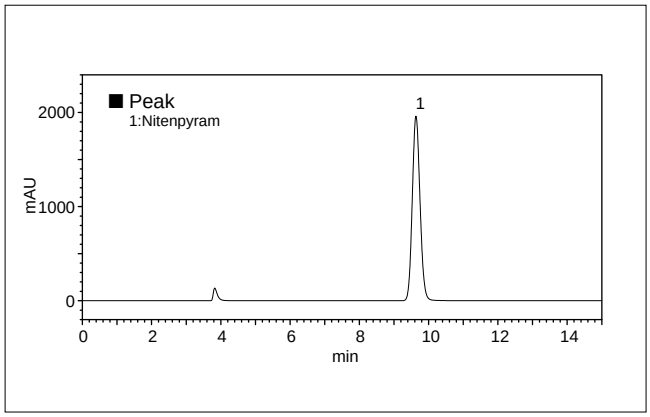


Fig. 1.13.3 Chromatogram of nitenpyram

■Analysis of Chlorfluazuron

Seven pesticides, including chlorfluazuron, can be analyzed simultaneously.

■Analytical Conditions

Column : Shim-pack VP-ODS (250mmL. × 4.6mm I.D.)
Mobile phase : Water/Acetonitrile = 3/7 (v/v)
Flow rate : 0.8mL/min
Temperature : 40˚C
Detection : SPD-10AV*VP* 250nm

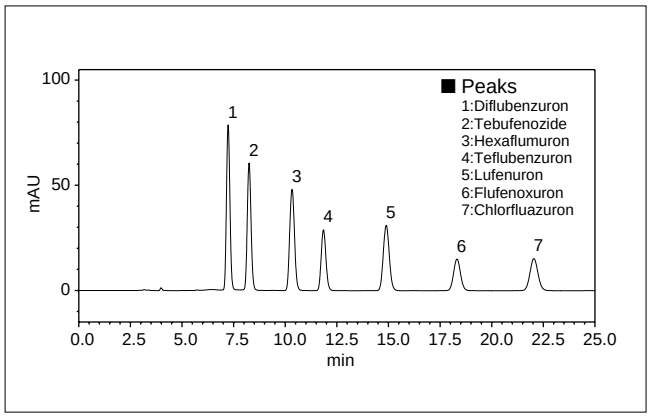


Fig. 1.13.4 Chromatogram of 7 pesticides including chlorfluazuron

1.14 GPC Clean-up Method Used in the Analysis of Pesticide Residue in Foods (1) - LC

■Explanation

In the analysis of pesticide residue in agricultural products, fats and pigments in the sample can cause contamination of the GC or GC/MS injection port and peaks that interfere with the target components and so they must be removed as part of the pretreatment process. The conventional solvent extraction method requires considerable time and effort and so difficulties arise when processing large numbers of samples.

GPC (gel permeation chromatography) is a technique that separates the sample components by molecular size. Using this technique, the pesticide components can be easily separated from the fats and pigments, which have relatively large molecular weights, and clean-up can be automated. For this reason, GPC is adopted as one of the clean-up methods in the pesticide-residue rapid analysis method prescribed by Japan's Ministry of Health, Labour and Welfare (Notice 43 issued by the Environmental Health Bureau's Food Chemistry Division on 8 April 1997).

The principle of the GPC clean-up method and an application example of Shimadzu's GPC Clean-up System are described here.

[References]

1) Committee for Studying and Developing the Pesticide-residue Rapid Analysis Method: Food Hygiene Research, Vol. 47, P35 (1997)

2) Isao Saito: LCtalk, Vol. 35, P3 (1995)

■Principle of GPC Clean-up Method

Fig. 1.14.1 shows the principle of the GPC clean-up method. There are small holes (pores) of a fixed size in the packing material of the GPC column. Components in the sample with a small molecular size (e.g., pesticides: gray sections in the figure) can permeate deep into the pores while constituents with a large molecular size (e.g., fats and pigments: striped sections in the figure) cannot. For this reason, fats and pigments are eluted from the column sooner than pesticides*) and so the sample can be purified by fractionating this pesticide eluate.

*)In practice, the separation process is not only affected by the molecular size but also by the adsorption onto the packing material.

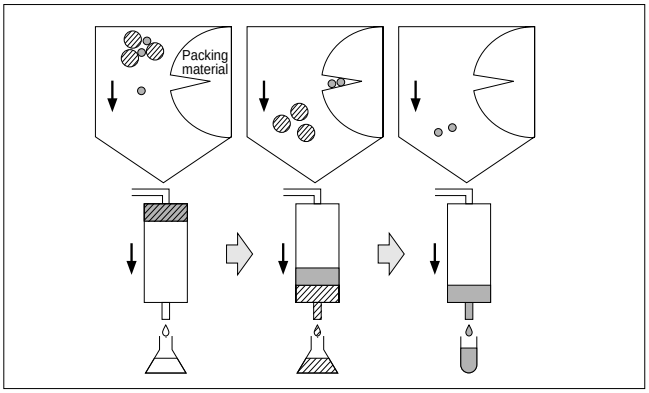


Fig. 1.14.1 Principle of GPC clean-up method

■Pretreatment for the Pesticide-residue Rapid Analysis Method

Fig. 1.14.2 shows the different stages in the pesticide-residue rapid analysis method. With the notified method (individual analysis method), fats and pigments were removed using liquid-liquid extraction and solid-phase extraction, whereas with the pesticide-residue rapid analysis method, they are removed using GPC. The pesticide-residue rapid analysis method makes it possible to perform pretreatment for all the pesticides together in almost the same amount of time required by the notified (individual analysis) method.

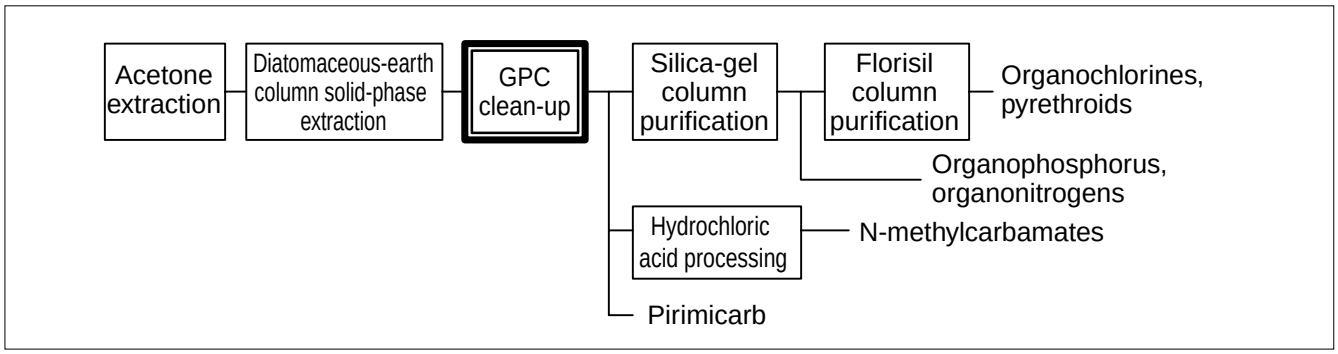


Fig. 1.14.2 Stages of pretreatment using the rapid analysis method

1.14 GPC Clean-up Method Used in the Analysis of Pesticide Residue in Foods (2) - LC

■Fractionation Conditions

The fractionation conditions for extracting a rice sample in accordance with the rapid analysis method and purifying it with Shimadzu's GPC Clean-up System are given in Table1.14.1. The corresponding chromatogram is shown in Fig. 1.14.3.

Fig. 1.14.3 also shows the chromatogram for two pesticides, fluvalinate and quinomethionate, obtained with GPC.

In general, the pesticides that are analyzed with the rapid analysis method are eluted between fluvalinate and quinomethionat and so fractionation is performed for the interval between the elution times of these two constituents.

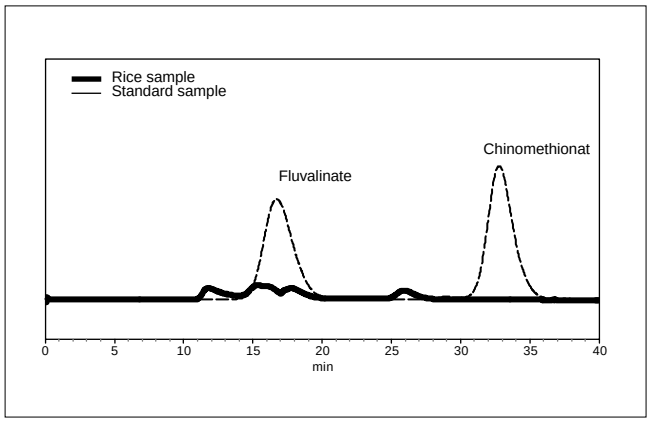


Fig. 1.14.3 GPC chromatogram of rice extract

Table 1.14.1 Fractionation Conditions

Instrument	: Shimadzu GPC Clean-up System
Column	: CLNpak EV-G+CLNpak EV-2000
Mobile phase	: A: Ethyl acetate B: Cyclohexane A/B = 1/4 (v/v)
Flow rate	: 4.0mL/min
Detection	: SPD-10A*VP* 254nm

■Analysis Example for Organophosphorus Pesticides

Fig. 1.14.4 shows the result obtained by purifying soybean, to which organophosphorus pesticides are added, using Shimadzu's GPC Clean-up System, purifying with a silica-gel mini-column, redissolving with acetone, and then analyzing the sample solution using GC.

Fig. 1.14.5 shows the result obtained by processing soybean, to which organophosphorus pesticides are added, in accordance with the notified (individual analysis) method, and analyzing with GC. It can be seen that almost identical results are obtained with both methods.

■Analytical Conditions

Instrument	: GC-2010
Column	: Rtx-1 (15m × 0.53mm I.D., df = 1.5µm)
Column Temp.	: 80˚C(1min)-8˚C/min - 250˚C(10min)
Inj. Temp.	: 230˚C
Det. Temp.	: 280˚C
Carrier Gas	: He, 16.5mL/min
Detection	: FPD-2010
Injection method	: Splitless (1min)

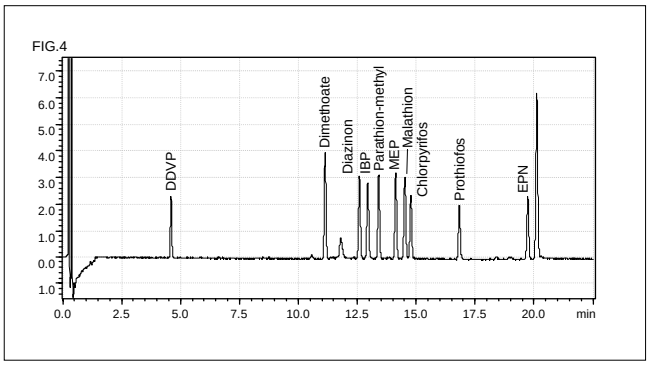


Fig. 1.14.4 Chromatogram of organophosphorus pesticides obtained using the rapid analysis method

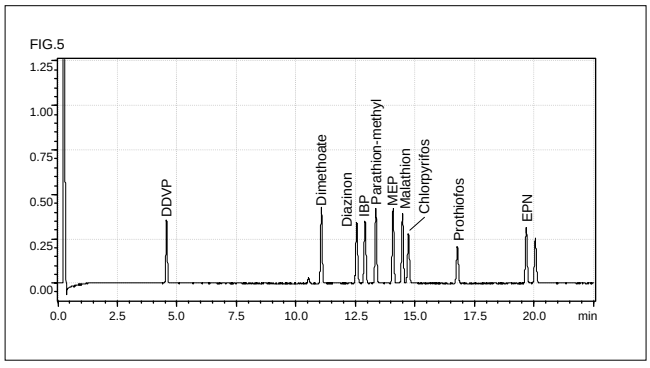


Fig. 1.14.5 Chromatogram of organophosphorus pesticides obtained using the notified (individual analysis) method

1.15 Analysis of Carbamate Pesticides - LC

■Explanation

N-methylcarbamate pesticides are used widely as insecticides and herbicides. In a publication issued by the Japanese government in 1994 (Ministry of Health, Labour and Welfare, Notice 199), post-column fluorescent derivatization using HPLC was adopted as the method for analyzing N-methylcarbamate pesticides.

N-methylcarbamate pesticides undergo hydrolysis in alkaline conditions and generate methylamine, which is a primary amine that can be analyzed by fluorescent detection after fluorescent derivatization.

■Analytical Conditions

[Separation]

Column	: Shim-pack FC-ODS (75mm × 4.6mm I.D.)
Mobile phase	: Water/Methanol (gradient elution method)
Flow rate	: 1.0mL/min
Temperature	: 50˚C

[Detection]

Reaction reagent 1	: 50mM NaOH
Flow rate	: 0.5mL/min
Temperature	: 100˚C
Reaction reagent 2	: OPA solution
Flow rate	: 0.5mL/min
Temperature	: 50˚C
Detection	: RF-10AXL Ex: 340nm Em: 445nm

■Rapid Analysis of N-methylcarbamate Pesticides

Fig. 1.15.1 shows the result of analyzing nine standard substances, including the N-methylcarbamate pesticides mentioned in Notice 199 issued by Japan's Ministry of Health, Labour and Welfare. By using the high-separation FC-ODS column as the analysis column, and optimizing the gradient program, methiocarbs, which are the slowest to elute, can be eluted in about 25 minutes. Because the column is shorter than those employed in conventional methods, gradient re-equilibration time and column cleaning time are also reduced. The time for one analysis cycle can be reduced to 32 minutes.

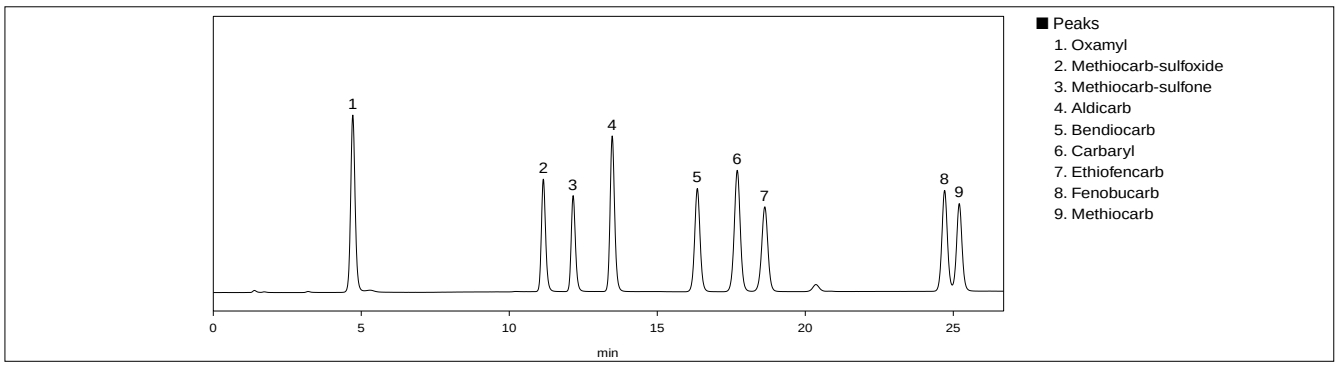


Fig. 1.15.1 Analysis of standard N-methylcarbamate pesticides (1ppm each, 10µL injected)

■High-sensitivity Analysis Example for N-methylcarbamate Pesticides

Fig. 1.15.2 shows the results of injecting 10µL of a sample with a concentration of 5ppb and performing high-sensitivity analysis.

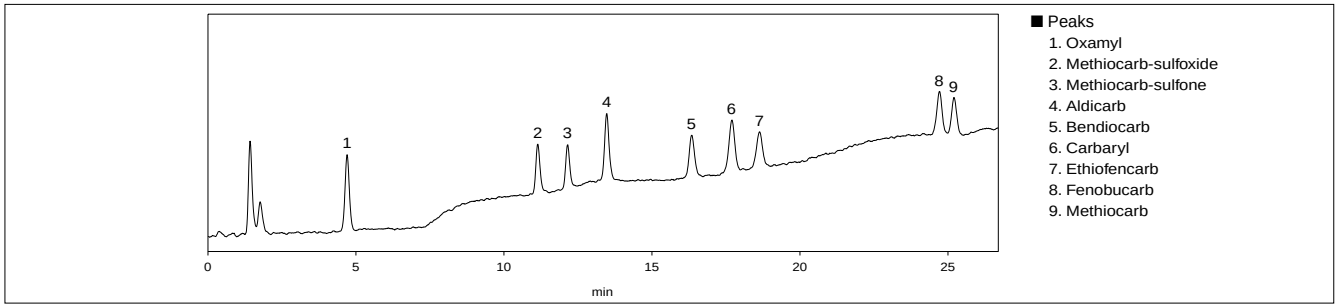


Fig. 1.15.2 Analysis of standard N-methylcarbamate pesticides (5ppm each, 10µL injected)

1.16 Analysis of Imazalil in Oranges - LC

■Explanation

Fungicide imazalil is mostly contained in imported oranges and bananas imported to Japan. Here, analysis of imported oranges will be introduced.
The target component was confirmed by comparison with UV spectrum of standard Sample using a photodiode array UV-VIS detector.

References

Shimadzu Application News No. L246 (C190-E068)

■Pretreatment

Performed in accordance with Standard Methods of Analysis for Hygienic Chemists, annotation (supplement 1995)

■Analytical Conditions

Column : STR ODS-II (150mmL. × 4.6mm I.D.)
Mobile phase: 5mM (Sodium) Phosphate Buffer(pH6.9) /Acetonitrile=45/55 (v/v)
Flow rate : 1.0mL/min
Temperature : 40°C
Detection : Photodiode array detection λ=210nm to 300nm

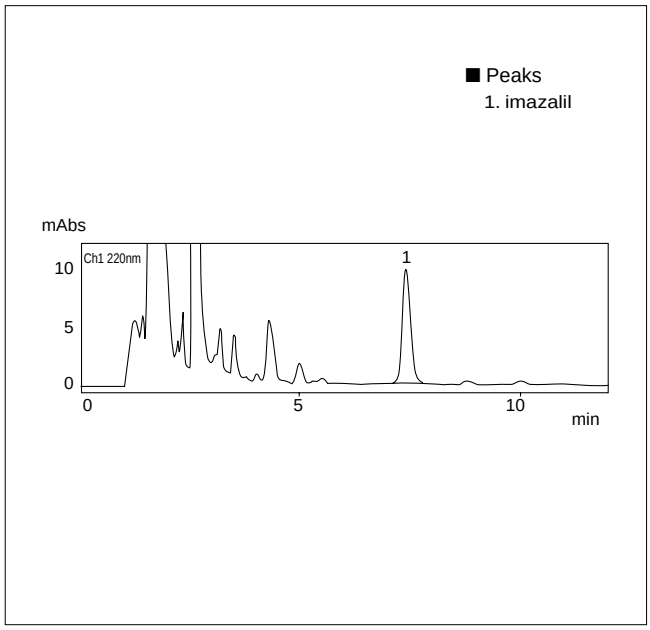


Fig. 1.14.1 Chromatogram of imazalil in imported orange sample (220nm)

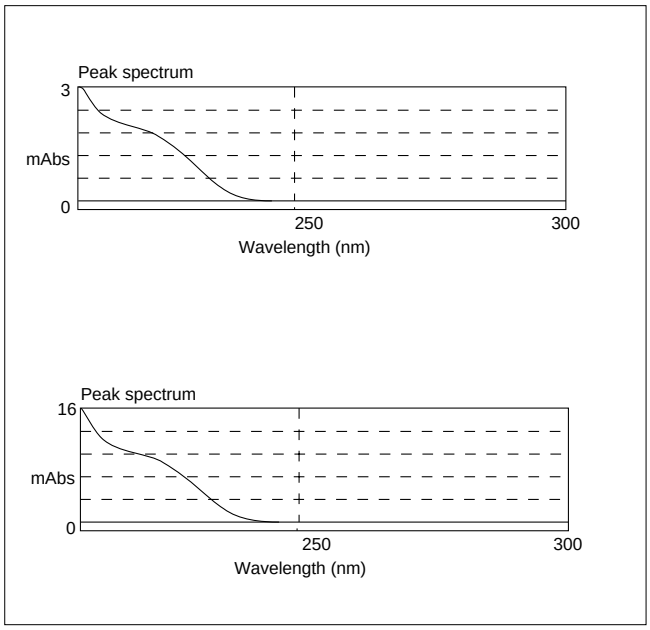


Fig. 1.14.2 Spectra of imazalil (upper: standard sample, lower: sample)

1.17 Analysis of Pesticide Residue in Agricultural Products Using LC/MS (1) - LC/MS

■Explanation

Under Japan's Food Sanitation Law, the levels of pesticide residue in agricultural products are strictly regulated, and at present there are 244 pesticides for 262 types of agricultural products. Out of the regulated pesticides, LC is used to analyze non-volatile pesticides or pesticides that are easily decomposed by heating. Because there are many impurities in food extracts, qualitative determination using LC/MS, which uses a mass spectrometer for detection and thereby offers greater selectivity, is increasingly employed for the purpose of verification.

The batch analysis of 20 residual pesticides in agricultural products is described here as an example. Fig. 1.17.1 shows a mass chromatogram obtained in scan mode. There is no need to perform optimization for each of the pesticides and high-sensitivity analysis is possible under the conditions set with autotuning. Also, if multi-sequence mode is used, reliable qualitative and quantitative determination is possible with one analysis by performing mass chromatography with positive ions or negative ions using the mass numbers of each of the pesticides.

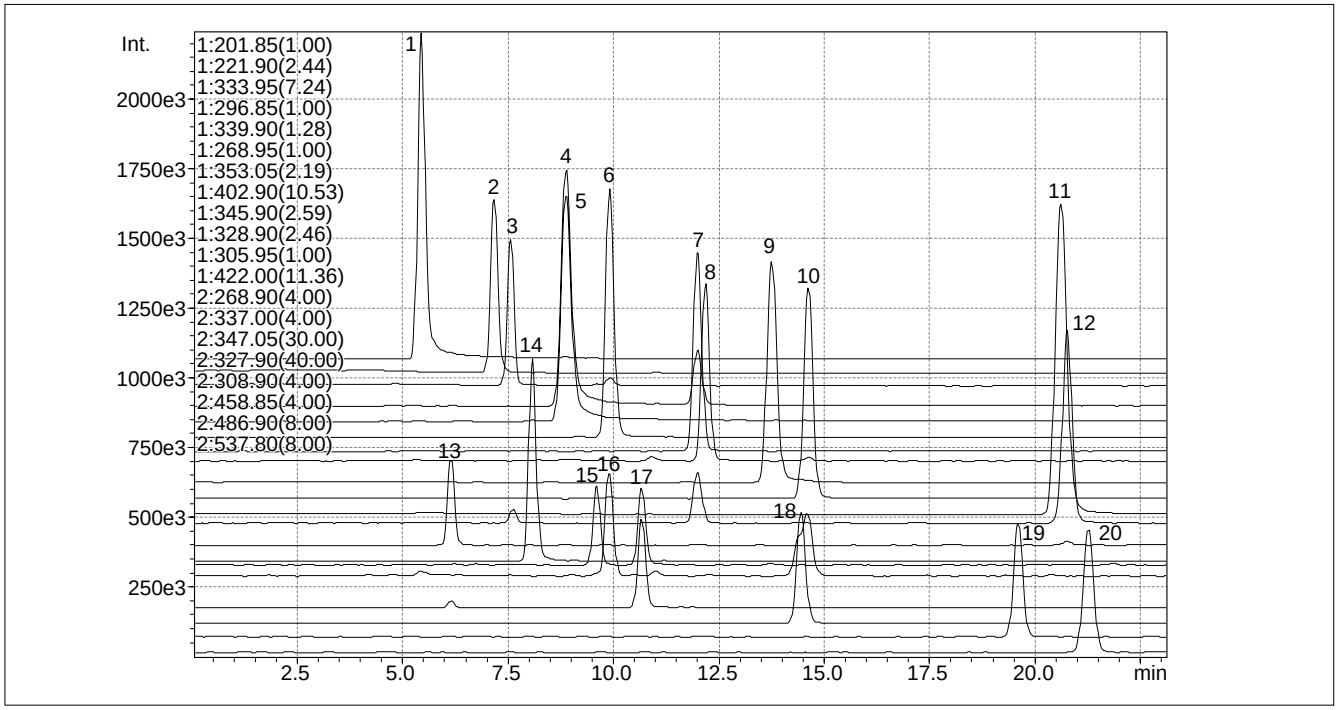


Fig. 1.17.1 Mass chromatogram of pesticides in agricultural products

■Peaks

ESI-Positive mode

1. thiabendazole MW 201
2. methabenzthiazuron MW 221
3. furametpyr MW 333
4. imazalil MW 296
5. etobenzanid MW 339
6. daimuron MW 268
7. tebufenozide MW 352
8. pyrazoxyfen MW 402
9. triflumizole MW 345
10. pencycuron MW 328
11. buprofezin MW 305
12. fenpyroximate MW 421

ESI-Negative mode

13. imibenconazole-debenzyl MW 270
14. inabenfide MW 338
15. myclobutanil MW 288
16. iprodione metabolite MW 329
17. diflubenzuron MW 310
18. hexaflumuron MW 460
19. flufenoxuron MW 488
20. chlorfluazuron MW 539

1.17 Analysis of Pesticide Residue in Agricultural Products Using LC/MS (2) - LC/MS

Fig. 1.17.2 shows SIM chromatograms and calibration curves (n=5) for pencycuron (12.5pg) and hexaflumuron (25pg) and Table 1.17.1 and 1.17.2 give the reproducibility results for each substance. As shown in this example, constituents with roughly the same retention times and different measurement modes (positive and negative ions) can be quantified at the same time.

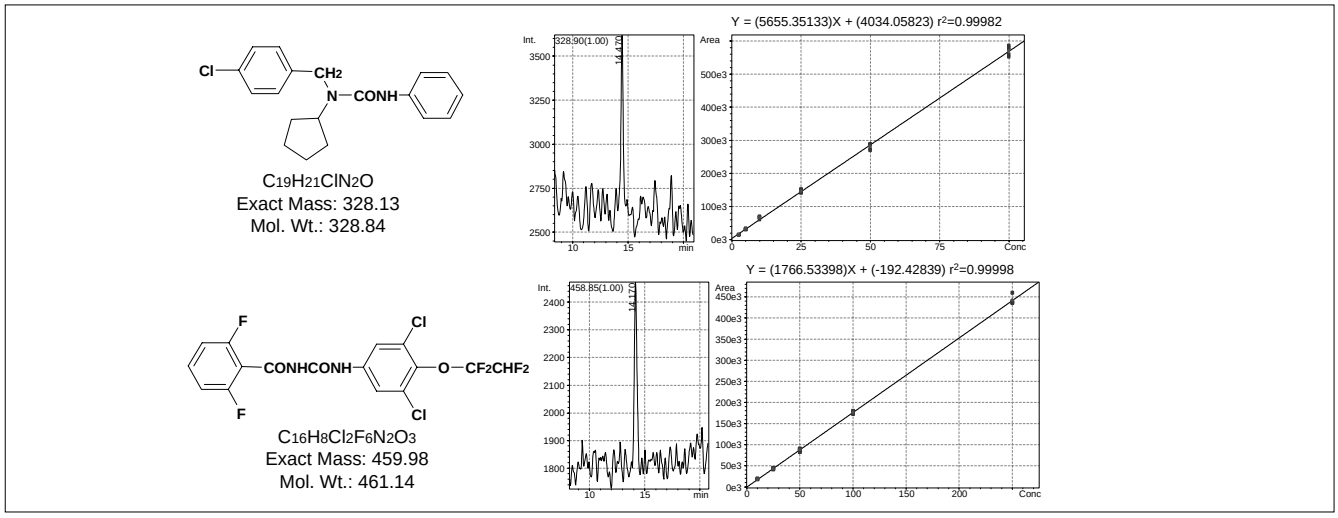


Fig. 1.17.2 SIM chromatogram and calibration curves for pencycuron and hexaflumuron

Table 1.17.1 Repeatability for pencycuron

	1	2	3	4	5	Average	Standard deviation	CV
12.5pg	13917	14526	14018	13948	15251	14332.00	570.19163	3.98 %
25pg	33710	33710	30242	30793	31331	31957.20	1645.7532	5.15 %
50pg	67238	68996	69932	66772	61325	66852.60	3346.1268	5.01 %
125pg	150565	145253	144468	152698	140439	146684.60	4929.2189	3.36 %
250pg	289289	287762	270265	288482	273127	281785.00	9281.106	3.29 %
500pg	586968	581783	560675	575145	551669	571248.00	14734.456	2.58 %

Table 1.17.2 Repeatability for hexaflumuron

	1	2	3	4	5	Average	Standard deviation	CV
25pg	11153	8984	9859	9766	9007	9753.80	883.07401	9.05 %
50pg	18690	19229	20473	18580	17762	18946.80	1001.644	5.29 %
125pg	42881	43726	40001	44840	43646	43018.80	1825.9509	4.24 %
250pg	81842	89280	90536	86530	91154	87868.40	3808.3029	4.33 %
500pg	174911	177600	180001	174125	172789	175885.20	2894.5713	1.65 %
1250pg	437000	437627	439882	434111	460184	441760.80	10502.585	2.38 %

Table 1.17.3 Analytical Conditions

Column	: Shim-pack VP-ODS (150mmL. × 2.0mm I.D.)
Mobile phase	: A: Water B: Acetonitrile
Gradient program	: 20% B → 60% B (0.03min) → 80% B (20min) → 100% B (20.01-30min) → 20% B (30.01-40min)
Flow rate	: 0.2mL/min
Column temperature	: 40˚C
Injection volume	: 5µL
Probe voltage	: +4.5kV (ESI-positive mode), -3.0kV (ESI-negative mode)
CDL temperature	: 200˚C
Block heater temperature	: 200˚C
Nebulizer gas flow rate	: 4.5L/min
CDL voltage	: +0V (ESI-positive mode), +0V (ESI-negative mode)
Q-array DC voltage	: Scan mode
Q-array RF	: Scan mode
Scan range	: m/z 50-650 (1.5sec/scan)

1.18 Analysis of N-methylcarbamate Pesticides Using LC/MS (1) - LC/MS

■Explanation

N-methylcarbamate pesticides are used widely in insecticides and herbicides and their residue in agricultural products has become an issue of concern. The method of separating eight N-methylcarbamate pesticides with an HPLC column, performing on-line hydrolysis, and applying fluorescent derivatization to the resulting methylamine is adopted as the testing method in revisions to the standards for foods and additives made by Japan's Ministry of Health, Labour and Welfare.

Here, however, the analysis of N-methylcarbamate pesticides directly using LC/MS in order to increase the simplicity and sensitivity, without applying derivatization, is described as an example.

Fig. 1.18.1 shows the structures and mass spectra of N-methylcarbamate pesticides. The protonated molecules can be confirmed in each case.

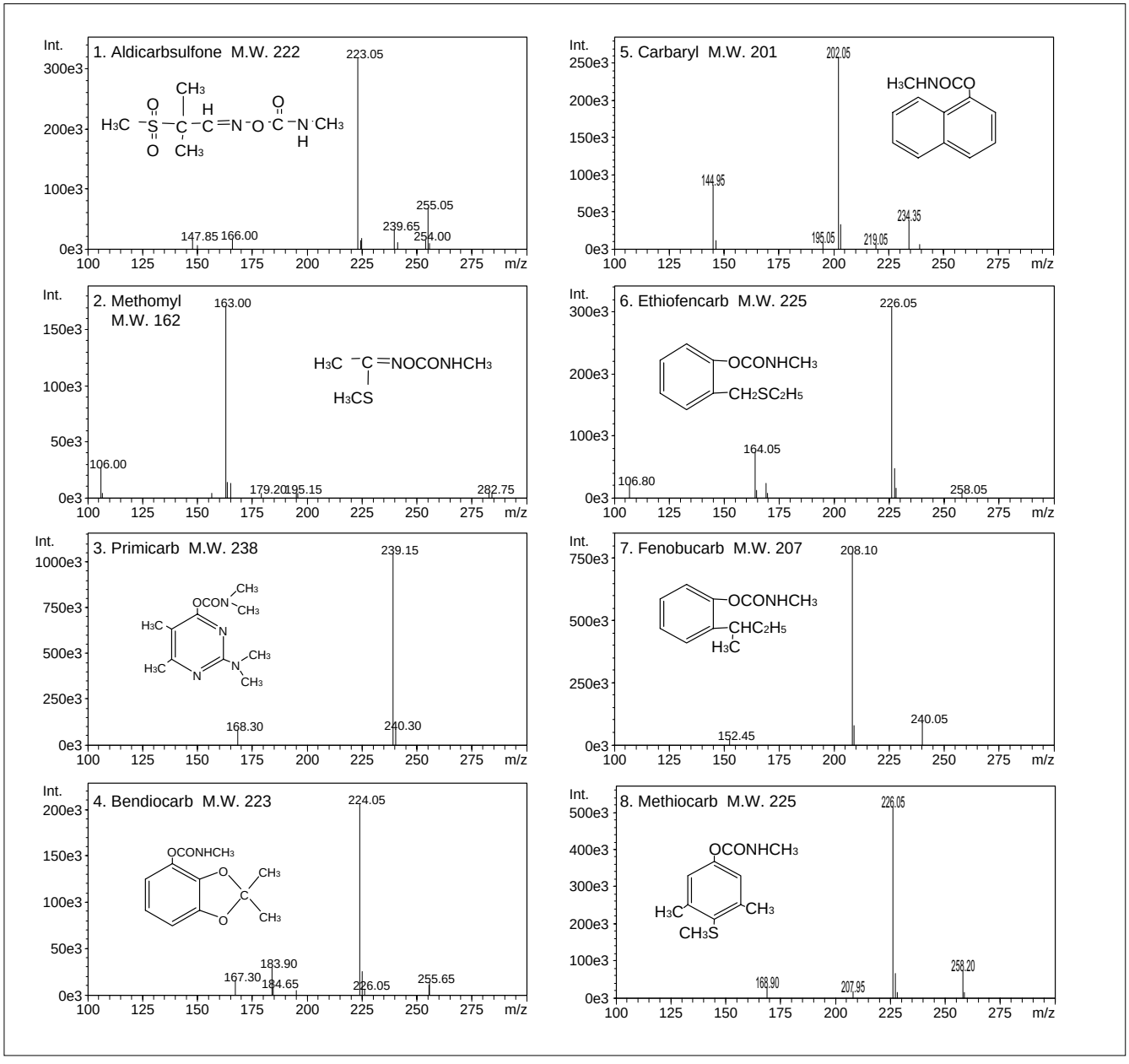


Fig. 1.18.1 Structures and mass spectra of N-methylcarbamate pesticides

1.18 Analysis of N-methylcarbamate Pesticides Using LC/MS (2) - LC/MS

Fig. 1.18.2 shows the chromatograms obtained when 5µL (5ng) of a 1ppm mixture of eight N-methylcarbamate pesticides is injected. The components that do not have clear peaks in the TIC chromatogram can be qualitatively analyzed easily by drawing mass chromatograms based on the characteristic mass numbers.

Fig. 1.18.3 shows the SIM chromatogram for fenobucarb at 40pg (8ppb, 5µL) and Fig. 1.18.4 shows the calibration curve (n = 5) between 40pg and 5ng. The CV values at different concentration are in the range 2% to 6%; highly precise results are obtained.

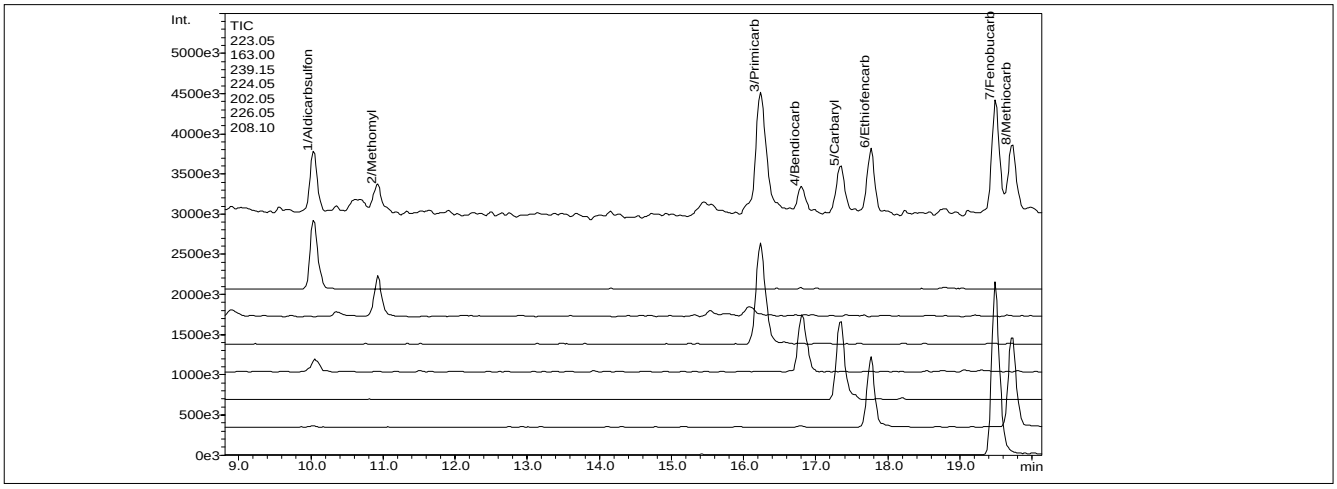


Fig. 1.18.2 TIC and mass chromatograms for N-methylcarbamate pesticides

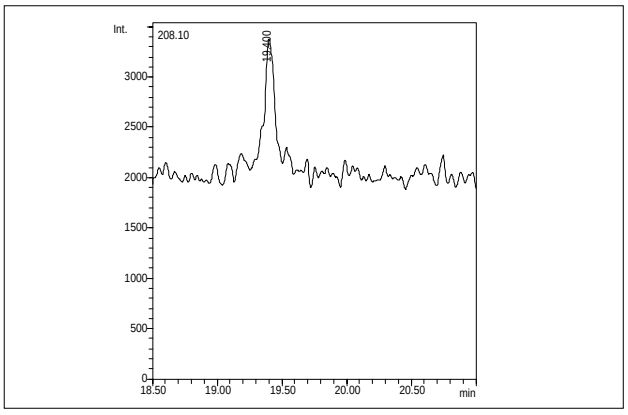


Fig. 1.18.3 SIM chromatogram of fenobucarb (40pg)

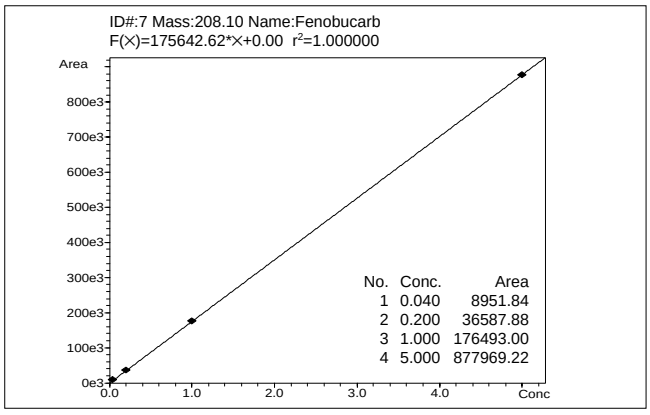


Fig. 1.18.4 Calibration curve for fenobucarb

Table 1.18.1 Analytical conditions

Column	: Shim-pack STR-ODS (150mmL. × 2.0mm I.D.)
Mobile phase	: A: 0.2% acetic acid solution B: Acetonitrile containing 0.2% acetic acid
Gradient program	: 0% B (0min) → 100% B (20min)
Flow rate	: 0.2mL/min
Column temperature	: 40˚C
Injection volume	: 5µL
Probe voltage	: +4.5kV (APCI-positive mode)
CDL temperature	: 230˚C
Probe temperature	: 200˚C
Nebulizer gas flow rate	: 2.5L/min
CDL voltage	: -30V
Deflector voltage	: +30V
Scan range	: m/z 100-400 (1.2sec/scan)

1.19 Analysis of Metribuzin Using Positive and Negative Ion Atmospheric Pressure Chemical Ionization (1) - LC/MS

■Explanation

Metribuzin, which is a triazine-group herbicide for annual weeds on potato, asparagus, and sugarcane fields, parks and roads, was included in an announcement made by the Japanese Environment Agency regarding the 67 types of chemical substances that are suspected of disrupting the endocrine system. Metribuzin has not yet been subjected to a thorough endocrinological investigation and so endocrine disruption has not been categorically established. It is, however, suspected of being an endocrine disruptor because of its reproductive toxicity and carcinogenicity.

In the 67 types of chemical compounds suspected of being endocrine disruptors, 44 are herbicides, insecticides, or germicides. Of these 44, there are 22 that are not registered or are invalid as pesticides in Japan. Metribuzin, however, is in active use and in order to investigate whether or not it is an endocrine disruptor, it is necessary to establish an easy analysis method and monitor metribuzin in the environment.

Japan's Food Sanitation Law specifies residue threshold levels not only for metribuzin itself but also for the combined total including its metabolites, desamino (DA), diketo (DK, methylthio-based desorption oxidant), and desaminodiket (DADK). The analysis of metribuzin and its metabolites using atmospheric pressure chemical ionization (APCI) LC/MS is described here as an example. Fig. 1.19.1 shows the structures of metribuzin and its metabolites.

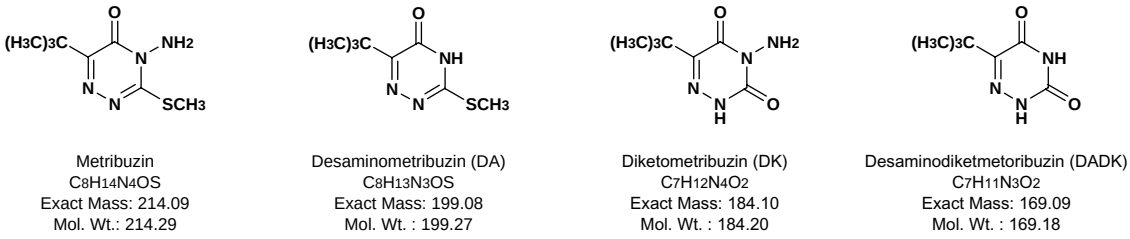


Fig. 1.19.1 Structures of metribuzin and its metabolites

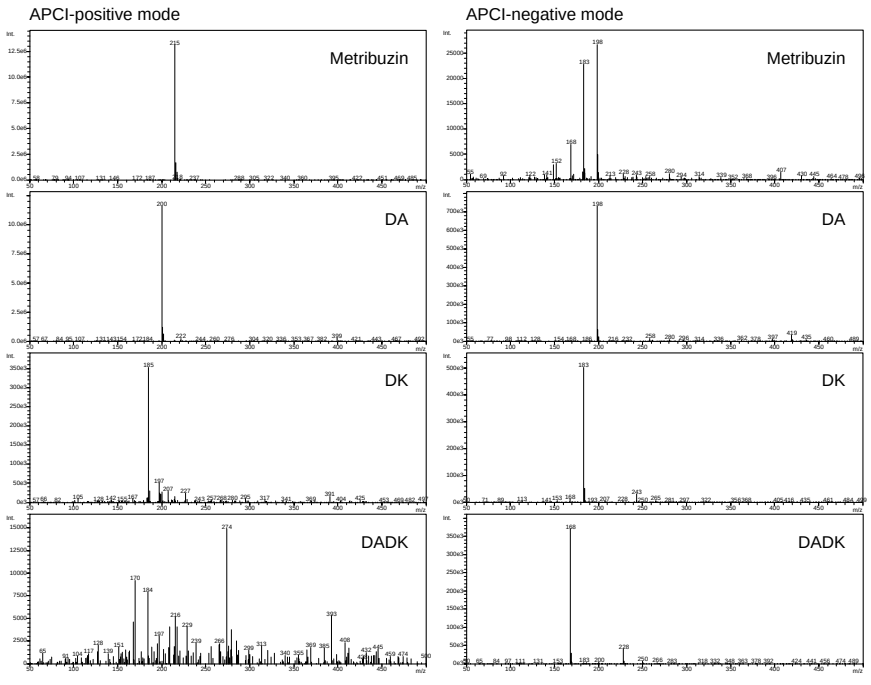


Fig. 1.19.2 Positive and negative APCI mass spectra of metribuzin and its metabolites

1.19 Analysis of Metribuzin Using Positive and Negative Ion Atmospheric Pressure Chemical Ionization (2) - LC/MS

In positive APCI mass spectra for metribuzin, DA, and DK, protonated molecules can be observed as standard peaks. The ion intensity is low for DADK protonated molecules (m/z 170) and the m/z 274 $(M-H + 2Na + AcOH)^+$ ion is observed instead. In negative APCI mass spectra, the $(M-H)^-$ molecular ion type can be observed as standard peaks for DA, DK, and DADK; metribuzin itself is observed as fragment ions m/z 199, 180, and 169, but the m/z 213 molecular ion type can hardly be observed. These results are thought to reflect the differences in the proton affinity of the compounds. In order to analyze metribuzin and its metabolites, however, the analysis of positive and negative ions is required. Fig. 1.19.3 shows the results of analyzing metribuzin and its metabolites using positive and negative ions. DK and DADK were detected with negative ions m/z 183 and 168, and metribuzin and DA were detected with positive ions m/z 215 and 200. The metribuzin, DA, DK, and DADK calibration curves (3.2 - 2,000 ppb, n = 5) showed good linearity at Y = 38312 X + 48246 (r^2 = 0.9998), Y = 3146 X + 21011 (r2 = 0.9999), Y = 1527 X + 3320 (r^2 = 0.9999), and Y = 1596 X + 1764 (r^2 = 0.9999) respectively, which means that highly accurate quantitative analysis is possible.

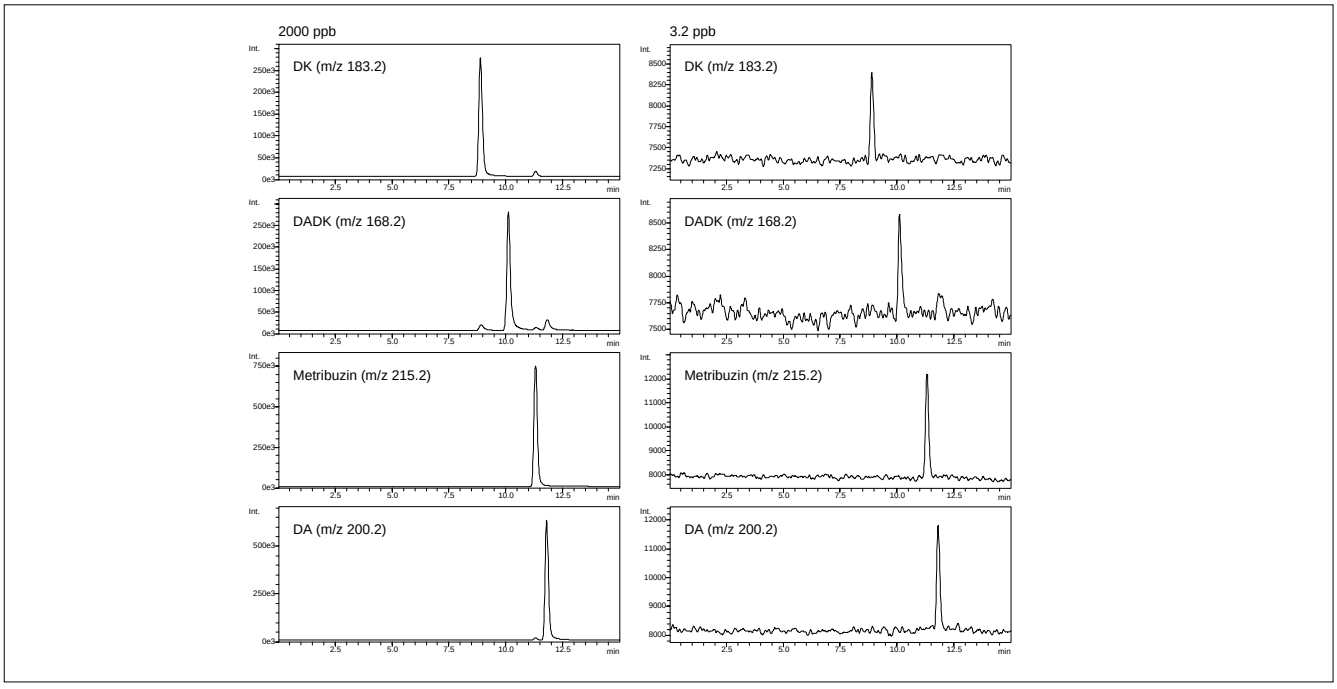


Fig. 1.19.3 Positive and negative SIM chromatograms of metribuzin and its metabolites (2,000ppb and 3.2ppb)

Table 1.19.1 Analytical Conditions

Column	: Insertsil ODS-2 (150mmL. × 2.1mm I.D.)
Mobile phase	: A: 0.2% acetic acid solution B: Methanol containing 0.2% acetic acid
Gradient program	: 30% B (0min) → 90% B (13-15min)
Flow rate	: 0.2mL/min
Column temperature	: 40˚C
Injection volume	: 50µL
Probe voltage	: +4.5kV (APCI-positive mode), -3.0kV (APCI-negative mode)
CDL temperature	: 230˚C
Probe temperature	: 400˚C
Nebulizer gas flow rate	: 2.5L/min
CDL voltage	: -30V (positive), +30V (negative)
Deflector voltage	: +30V (positive), -20V (negative)
Scan range	: m/z 215.2, 200.2 (positive), m/z 183.2, 168.2 (negative)

1.20 Analysis of Phenoxypropionic-acid Herbicides Using LC/MS (1) - LC/MS

■Explanation

Fluazifop, quizalofop-butyl, and other phenoxypropionic-acid herbicides are used widely throughout the world because they have strong herbicidal effects at low doses. The active substance is carboxylic acid-based and inhibits the biosynthesis of fatty acids by acetyl-CoA carboxylase inhibition. In Japan, the residue of these herbicides is an issue of concern and official testing methods for quizalofop-ethyl, cyhalofop-butyl, and fluazifop have been established. The total amount of quizalofop is measured using HPLC or LC/MS after hydrolysis of quizalofop-ethyl, and the total amount of fluazifop is measured by performing GC or GC/MS on the esters formed from butyl esterification after hydrolysis.

The batch analysis of fluazifop, fluazifop-butyl, quizalofop, and quizalofop-ethyl using electrospray ionization (ESI) is described here. The ESI method effectively ionizes carboxylic acid-based herbicides (fluazifop and quizalofop) with negative ions and the ester-based herbicides (fluazifop-butyl and quizalofop-ethyl) with positive ions. Fig. 1.20.1 shows the mass spectra of these compounds. The deprotonated molecules $(M-H)^-$ of the carboxylic acid-based herbicides in negative ion mode and the protonated molecules $(M+H)^+$ of the ester-based agricultural chemicals in positive ion mode can be confirmed.

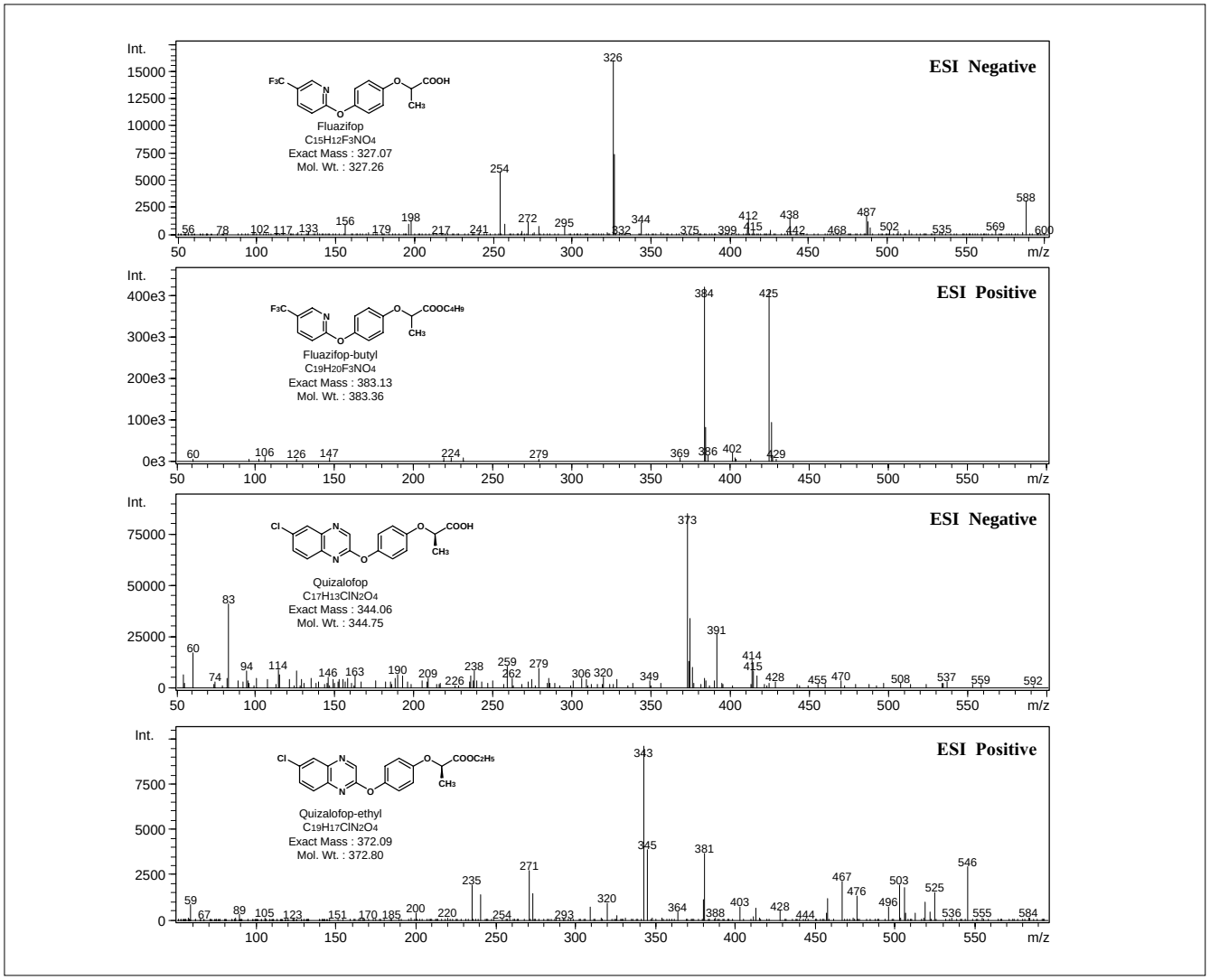


Fig. 1.20.1 ESI mass spectra of phenoxypropionic-acid herbicides

1.20 Analysis of Phenoxypropionic-acid Herbicides Using LC/MS (2) - LC/MS

When using a reversed-phase column, the retention time of ester-based herbicides is longer than that of carboxylic acid-based herbicides. Therefore, it is possible to analyze both carboxylic acid-based and ester-based herbicides at the same time by first selecting negative ion detection, and then, after the elution of carboxylic acid-based herbicides is complete, switching to positive ion detection (Fig. 1.20.2). Good calibration curves were produced for each substance at concentrations in the range 0.8ppb to 500ppb. Fig. 1.20.3 shows the calibration curve and SIM chromatogram at 0.8ppb for fluazifop.

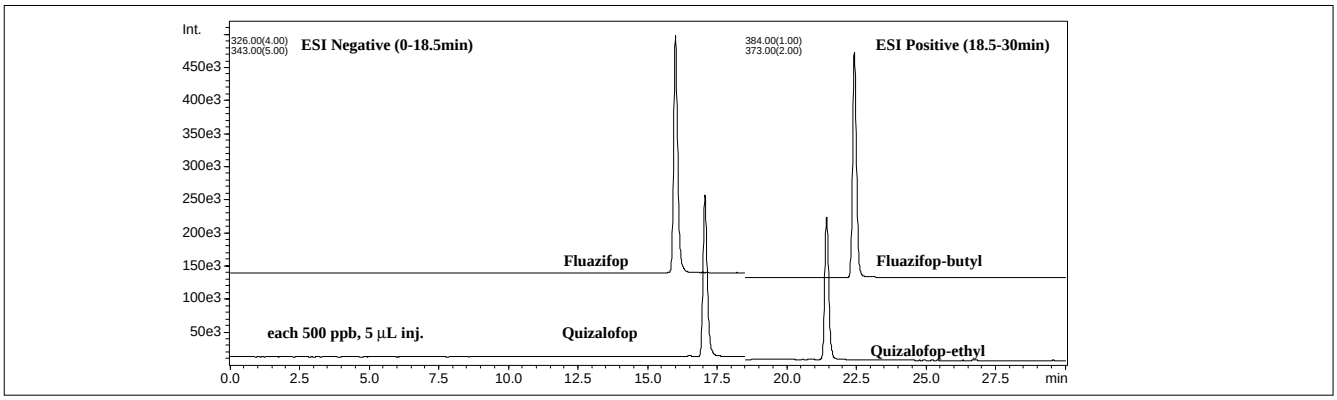


Fig. 1.20.2 ESI mass chromatograms of phenoxypropionic-acid herbicides

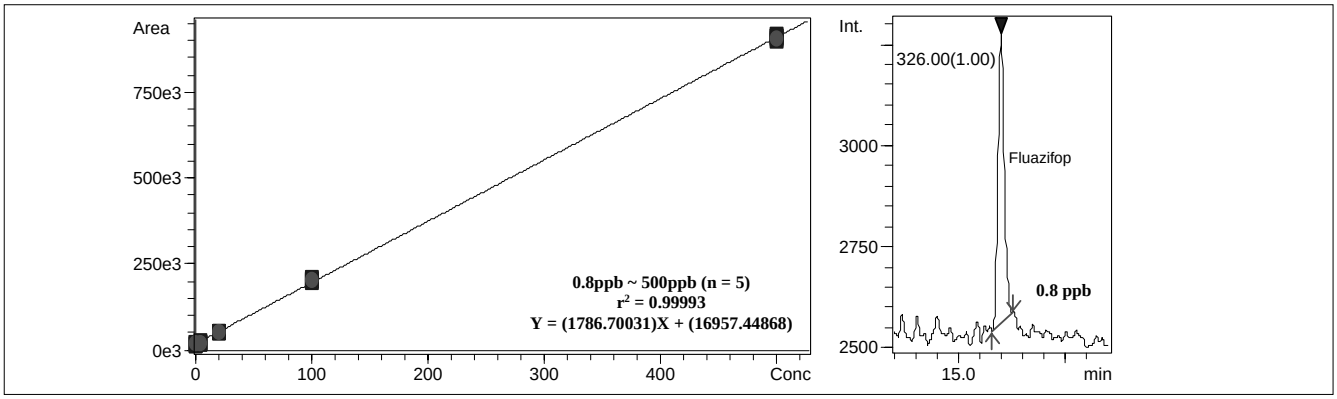


Fig. 1.20.3 Calibration curve and SIM chromatogram of fluazifop

Table 1.20.1 Analytical Conditions

Column	: Shim-pack VP-ODS (150mmL. × 2.0mm I.D.)
Mobile phase	: A: 0.1% formic acid solution B: Acetonitrile containing 0.1% formic acid
Gradient program	: 20% B (0min) → 90% B (20-30min)
Flow rate	: 0.2mL/min
Column temperature	: 40˚C
Injection volume	: 5µL
Probe voltage	: -3.5kV (ESI- negative mode), +4.5kV (ESI-positive mode)
CDL temperature	: 200˚C
Block heater temperature	: 200˚C
Nebulizer gas flow rate	: 4.5L/min
Q-array DC voltage	: -30V, 10V
Q-array RF voltage	: 150
Scan range	: m/z 50-600 (1.0sec/scan)
SIM	: m/z 326, 343, 384, 373 (0.5sec/ch)

2.1 Analysis of Antibiotics and Synthetic Antibacterial Agents in Livestock and Farm-raised Fish (1) - LC

■Explanation

When livestock and farm-raised fish are given drugs, the quantity of residual drugs remaining in the body varies depending on the rate the drugs are absorbed and excreted. In general, the larger the quantity of drugs given, the more residual drugs will remain in the body. However, when comparing different drugs, the residual amount is determined by the amount absorbed and the amount excreted, which depend on the physical characteristics of the drug.

The Food Sanitation Law and related regulations in Japan stipulate that any residual drug must not be detected. However, the detection limits of residual drugs, which were in the order of ppm, have lowered to the order of ppb or ppt due to advances in analytical instruments and methods. Accordingly, inspection methods with lower detection limits have been stipulated, where the drugs are regarded as "not detected" if their detected values are below specified detection limits. As a guideline, the limit values are in the order of 20 to 30 ppb.

HPLC is an essential instrument for analyzing synthetic antibacterial agents and antibiotics due to its excellent sensitivity and reproducibility, as well as the ease of pretreatment. This example shows analysis data for drugs often used for poultry and farm-raised eels.

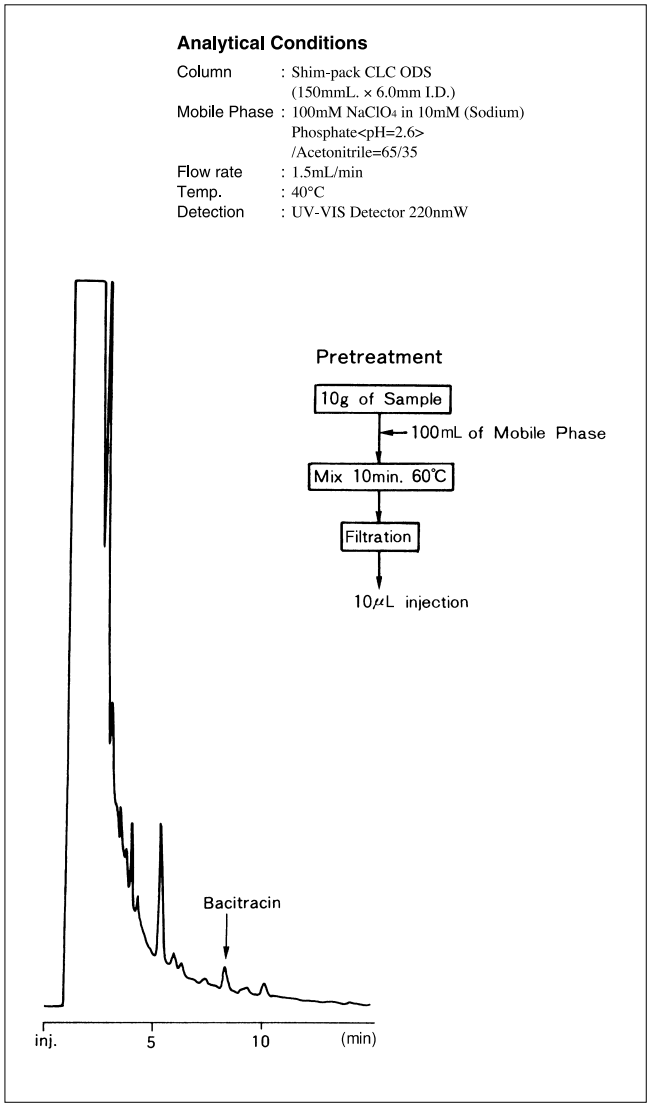


Fig. 2.1.1 Analysis of Bacitracin in Poultry Feed

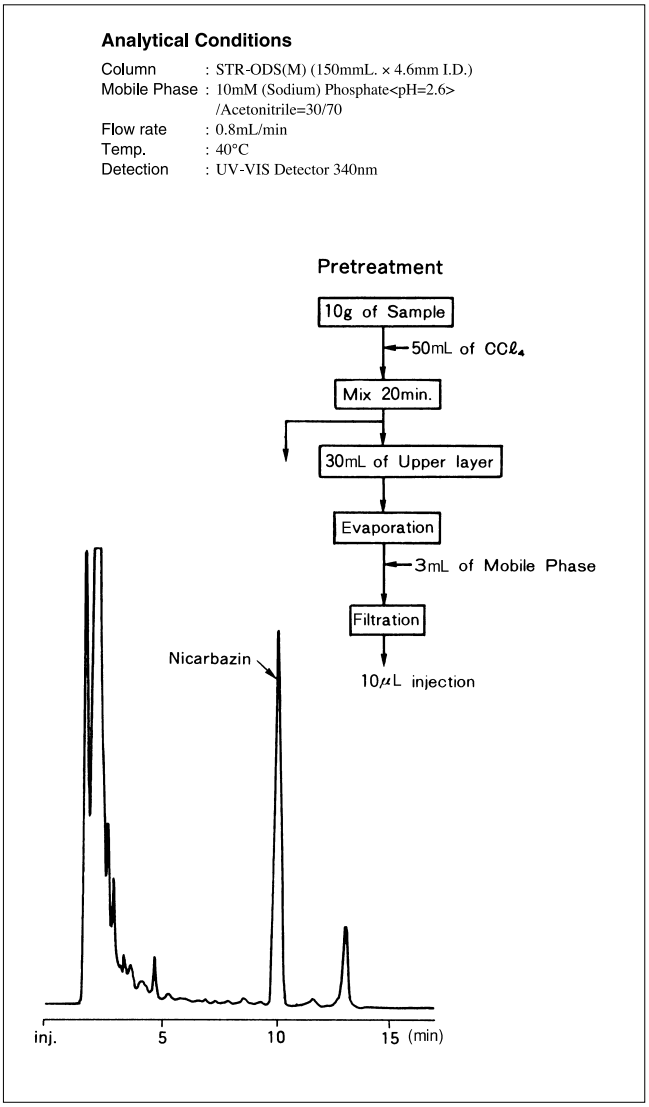


Fig. 2.1.2 Analysis of Nicarbazin in Poultry Meat

2.1 Analysis of Antibiotics and Synthetic Antibacterial Agents in Livestock and Farm-raised Fish (2) - LC

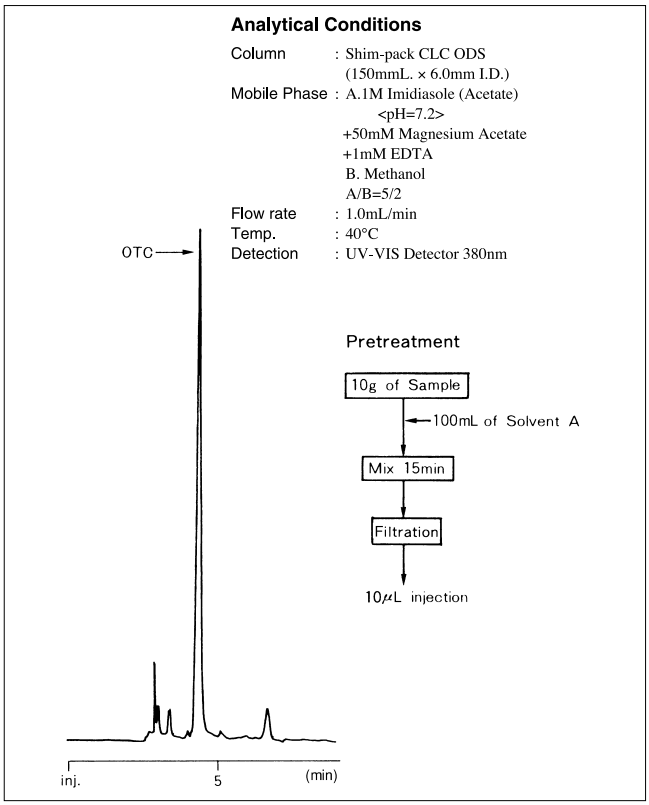


Fig. 2.1.3 Analysis of Oxy-tetracycline in Eel

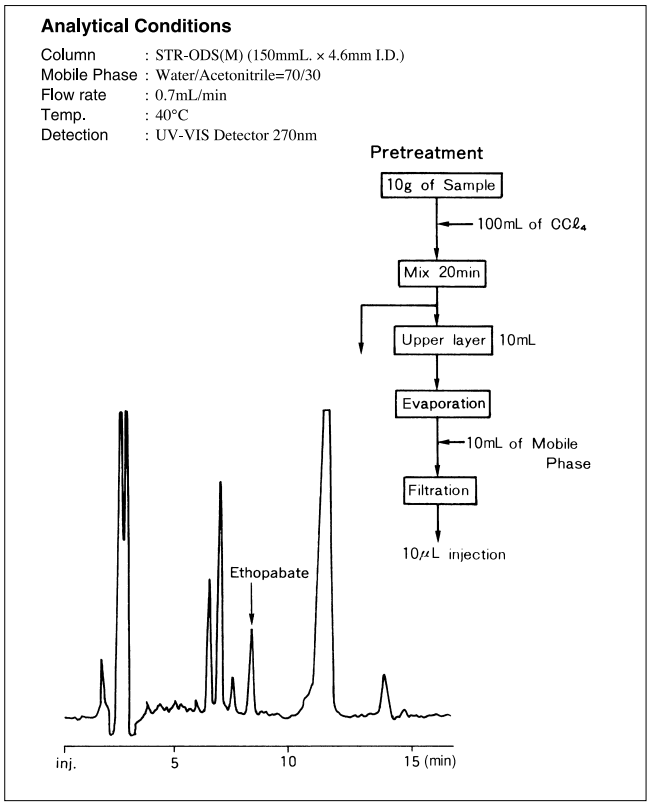


Fig. 2.1.4 Analysis of Ethopabate in Eel

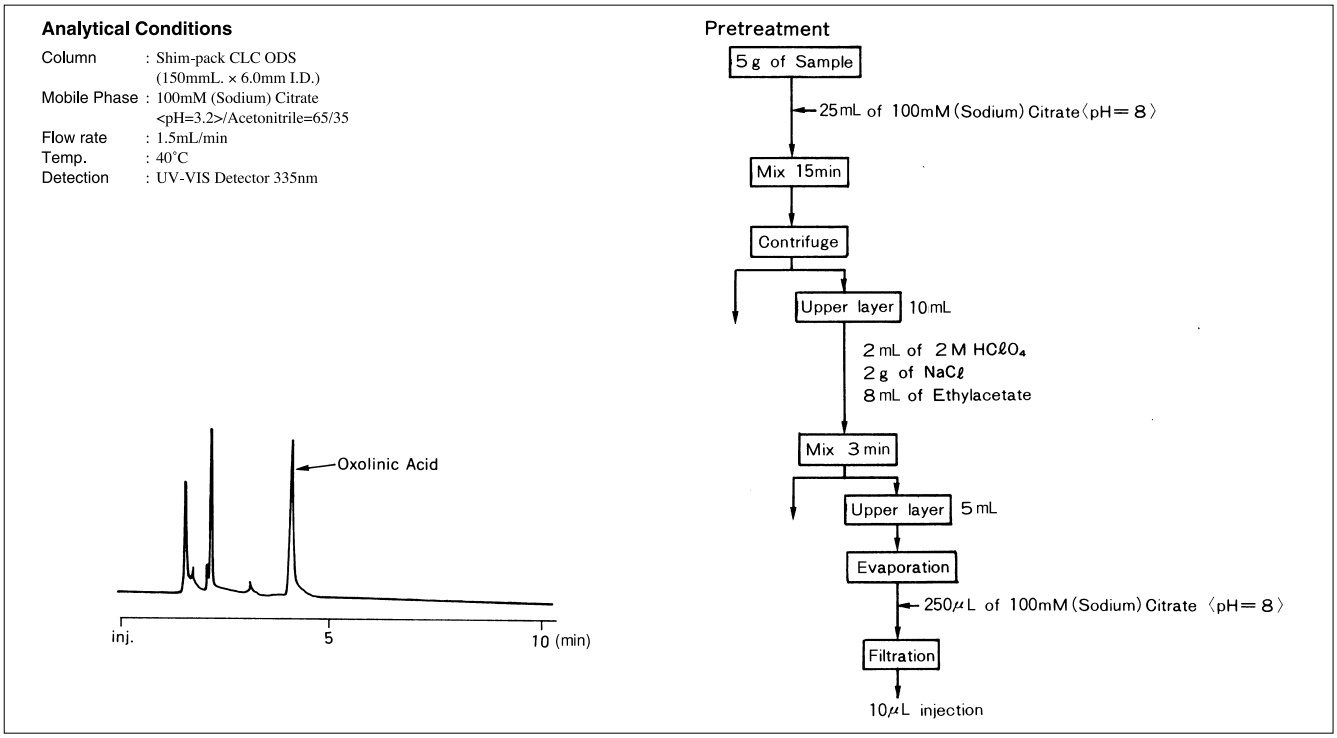


Fig. 2.1.5 Analysis of Oxolinic Acid in Eel

2.2 Analysis of Oxytetracycline - LC

■Explanation

Smple was extracted from shop-sold pig liver using the official gazette method and oxytetracycline was added to make a solution of 0.5ppm for analysis.

References

Official Gazette extra No. 245 (December 26, 1995)

■Pretreatment

The sample was pre-treated as shown in Table 2.2.1 in accordance with the official gazette.

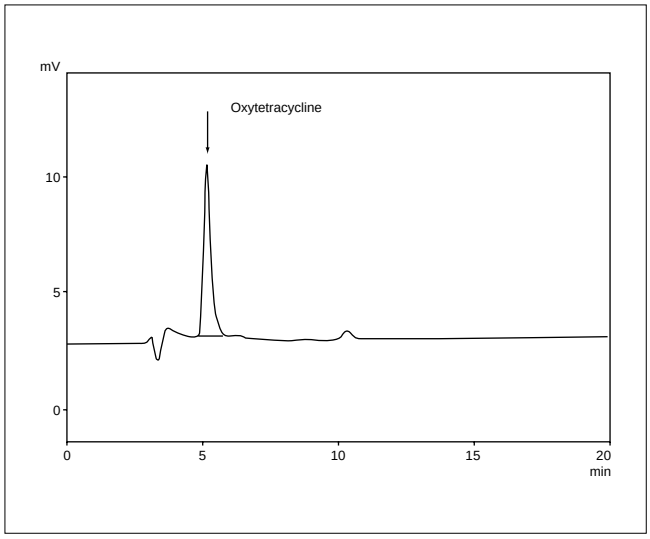


Fig. 2.2.1 Analysis example of oxytetracycline

■Analysis of Oxy-tetracycline in Beef

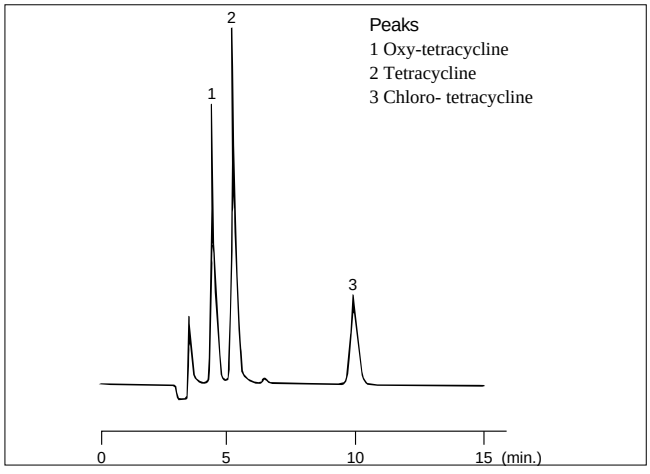


Fig. 2.2.3 Analysis of Oxy-tetracycline in Beef

■Analytical Conditions

Column : STR ODS-II (150mm. × 4.6mm I.D.)
Mobile phase : 1M Imidazole Buffer/Methanol=77/23 (v/v)
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : Fluorescence detector
Ex380nm Em520nm

■References

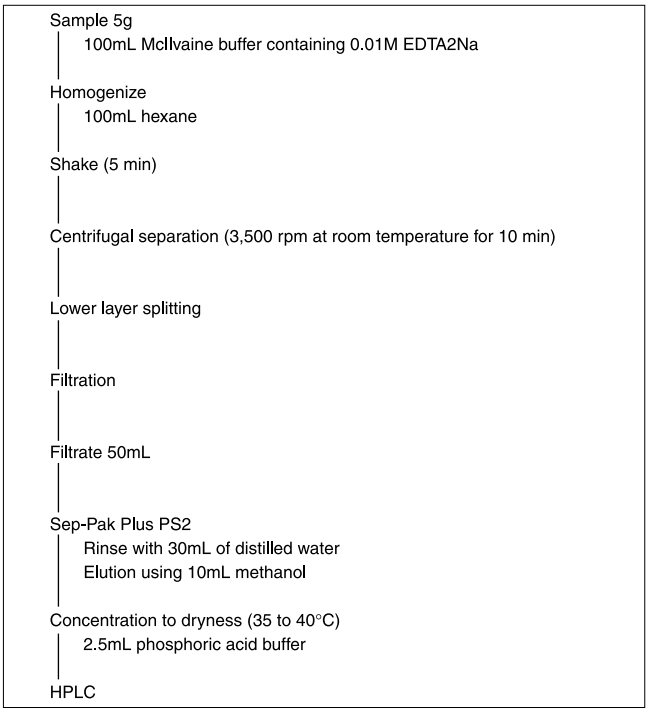


Fig. 2.2.2 Pretreatment flowchart for oxytetracycline

■Analytical Conditions

Column : Shim-pack CLC-ODS
(150mmL. × 6.0mm I.D.)
Mobile phase : A / B=7 / 3 (v / v)
A: 1M (Aceticacid) imidazole buffer (pH 7.2) containing 50mM magnesium acetate, and 1mM ECTA
B: Methanol
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : Fluorescence detector (Ex 380nm Em 520nm)

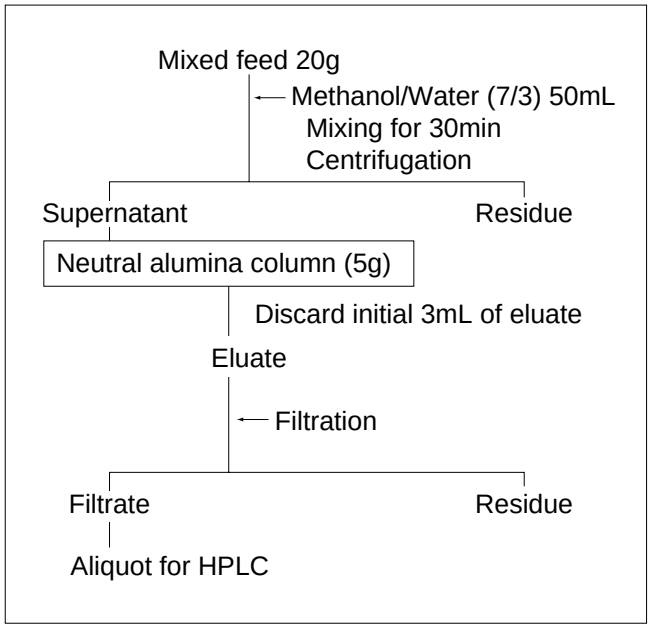
2.3 Analysis of Olaquindox in Mixed Feed - LC

■Explanation

Olaquindox is an additive used in mixed feed for piglets, etc., and works to promote the effective use of nutritious elements contained in feed. The example shows olaquindox separated using reversed-phase chromatography (Fig. 2.3.1). This substance has absorption peaks in the region of 270nm and 380nm (Fig. 2.3.2), of which 270nm shows higher sensitivity, but 380nm has better selectivity. Detection was performed at 380nm in this analysis.

■Pretreatment

This shows the pretreatment for the sample. Light must be shutout during operation because olaquindox easily changes in light.



■Analytical Conditions

Column : Shim-pack CLC-ODS
(150mmL. × 6.0mm I.D.)
Mobile Phase : A : 10mM Sodium Phosphate Buffer (pH2.6)
: B : Methanol
A : B=7 : 3 (v/v)
Guard Column : Shim-pack G-ODS (4) (10mmL × .4.0mm I.D.)
Column temp. : Room Temp.
Flow rate : 0.7mL/min
Detection : UV-VIS Detector 380nm

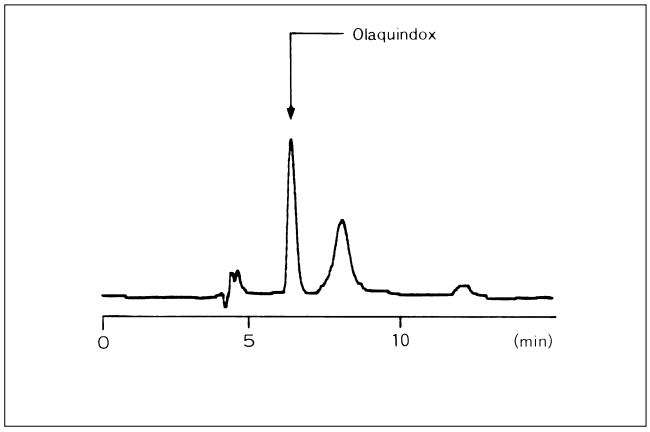


Fig. 2.3.1 Analysis example for mixed feed

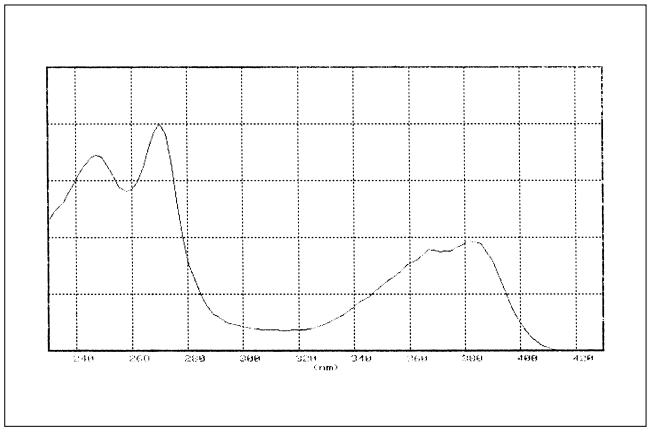


Fig. 2.3.2 Spectrum of standard

2.4 Analysis of Lasalocid in Mixed Feed - LC

■Explanation

Lasalocid is a polyether group antibiotic added to feed for broiler chicks with the aim of preventing coccidiosis. However, if excessive amounts of this substance are given to hens, it can cause development disability, so feed producers are obligated to control this.

This data shows an example of lasalocid contained in broiler chick mixed feed that has been separated using reversed-phase chromatography. Detection was performed using both ultraviolet absorption and fluorescence detectors. The fluorescence detector is more effective for sensitivity and selectivity.

■Pretreatment

Sample pretreatment involves extraction with chloroform, followed by adsorption in a silica gel column and elution with methanol. However, in the case of samples containing a lot of lasalocid, such as premix, they are more conveniently prepared by extracting the lasalocid with methanol, filtering it, and injecting that filtrate as it is.

■Analytical Conditions

Column : Shim-pack CLC-ODS (150mmL. × 6.0mm I.D.)

Mobile Phase : Methanol/Water = 9/1(v/v) containing 100mM $NaClO_4$

Guard Column : Shim-pack G-ODS (4) (10mmL. × 4.0mm I.D.)

Column temp. : 40°C

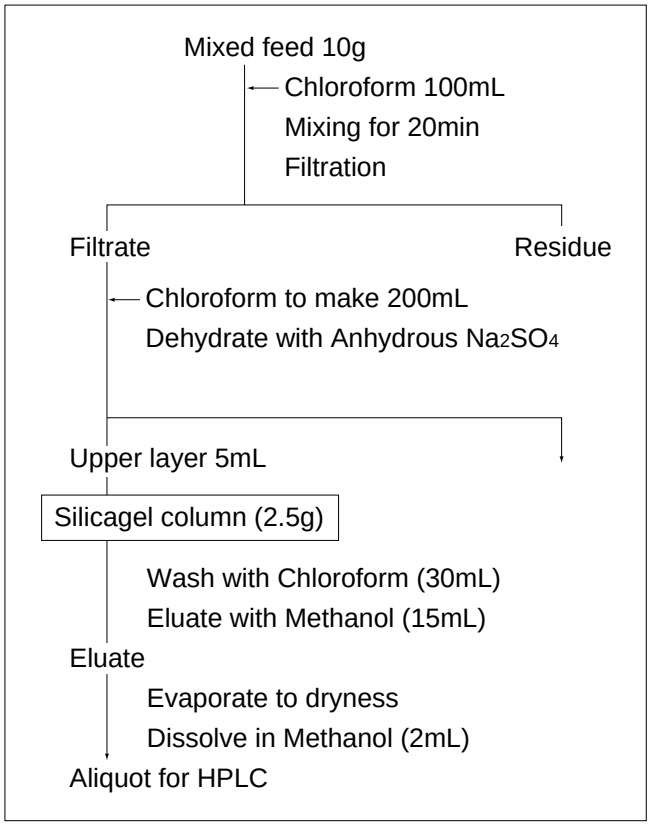
Flow rate : 1.0mL/min

Detection : UV-VIS Detector 310nm

Fluorescense Detector Ex : 310nm, Em : 425nm

References

Mixed feed containing lasalocid was provided by Marubeni Shiryo Corporation.



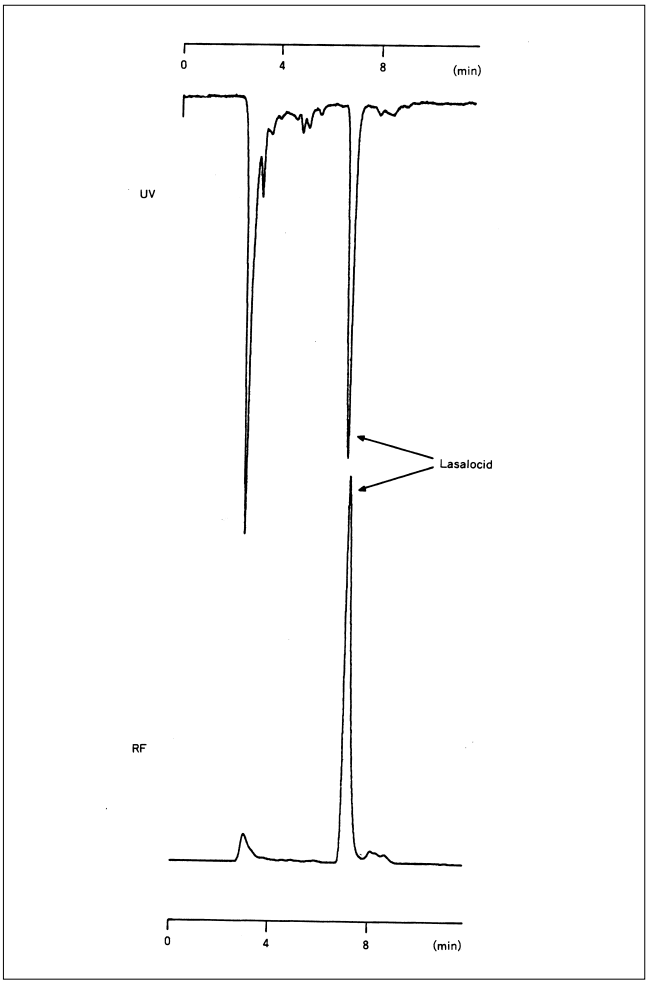


Fig. 2.4.1 Analysis example of mixed feed

2.5 Analysis of Polyether Antibiotics in Animal Feeds (1) - LC

■Explanation

The Japanese Ministry of Agriculture, Forestry and Fisheries designates polyether antibiotics salinomycin sodium and monensin sodium as animal feed additives for enhancing the effectiveness of nutrients contained in animal feeds. These substances contained in poultry and bovine feeds were conventionally analyzed by microbiotic quantitation in accordance with the Animal Feed Analysis Standards. However, as this method requires two days for results to be obtained, faster quantitative methods were being pursued.

Given this situation, the Animal Feed Analysis Standards were partially revised as of April 10, 2002, to incorporate LC post-column derivatization method to analyze salinomycin sodium and monensin sodium.

■Detection Method

Salinomycin sodium and monensin sodium produce color when heated with vanillin (4-hydroxy-3-methoxybenzaldehyde) in sulfuric acid and methanol. This reaction is known as a Komarowsky reaction, and this post-column derivatization system uses the Komarowsky reaction. Polyether antibiotics narasin and semduramycin are also analyzed by the same method.

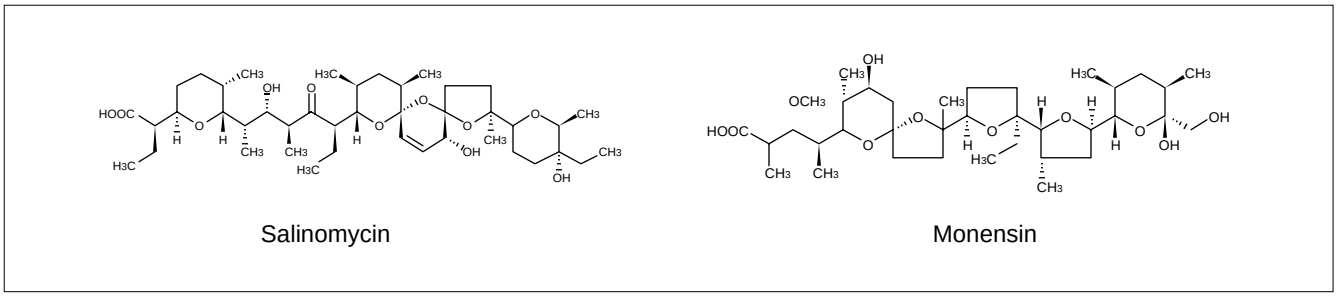


Fig. 2.5.1 Salinomycin and Monensin Structures

Fig. 2.5.2 shows the flow diagram for this system. A vanillin reagent is continuously added to the polyether antibiotics that were separated in the reversed-phase column, and the target substances are detected with a visible absorption detector (520 nm) after being heated at 95°C in the reaction chamber.

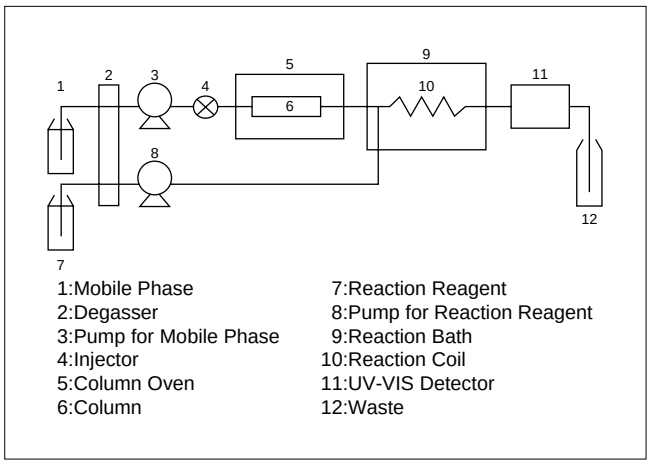


Fig. 2.5.2 System Flow Diagram

2.5 Analysis of Polyether Antibiotics in Animal Feeds (2) - LC

■Analysis of Standard Samples

Fig. 2.5.3 shows an example of analyzing a mixture of standard monensin sodium, salinomycin sodium and narasin samples*. The sample mixture concentration was 10 mg/L (methanol : water = 9:1 solution) for each substance and 10µL of the mixture was injected. The substances were easily separated under these analytical conditions.

* Standard samples are from the Fertilizer and Feed Inspection Station, Japan

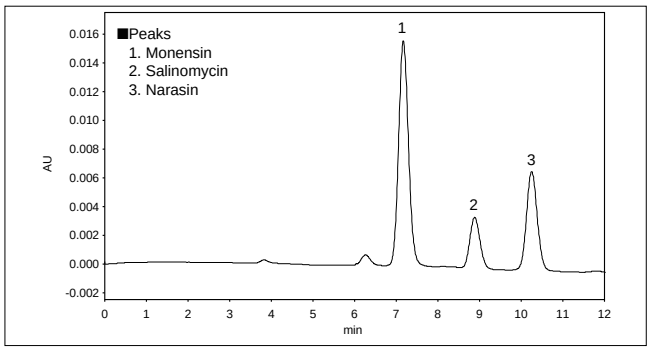


Fig. 2.5.3 Chromatogram for Standard Samples

■Analysis Conditions

Column	: Shim-pack FC- ODS (150mmL. × 4.6mm I.D.)
Mobile Phase	: Methanol/Water/Acetic Acid=940/60/1(v/v/v)
Flow Rate	: 0.6mL/min
Column Temp	: 40˚C
Reaction Reagent	: Methanol/Sulfuric Acid/Vanillin=95/2/3(v/v/v)
Reaction Temp	: 95˚C
Detection	: VIS at 520nm

■Analysis of Animal Feed

Fig. 2.5.5 shows an example of analyzing monensin sodium added to animal feed. After extraction by agitation in methanol:water = 9:1 solution and filtering, 10µL of the filtrate was injected. Salinomycin sodium and narasin added to animal feed can also be analyzed without the affection of interfering substances by the same extraction method.

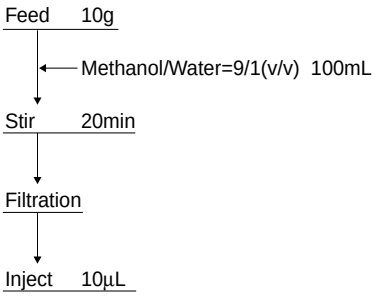


Fig. 2.5.4 Pretreatment

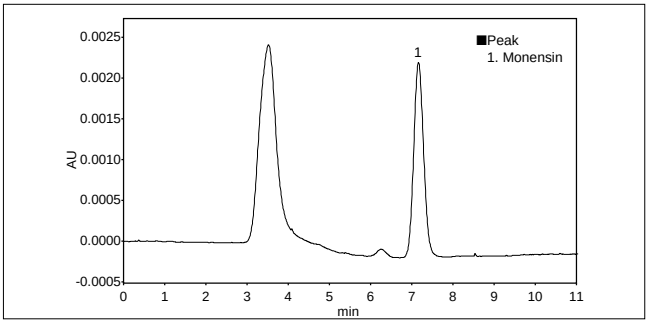


Fig. 2.5.5 Chromatogram of Animal Feed

2.6 Analysis of Spiramycin - LC

■Analysis of Spiramycin

When spiramycin is metabolized by livestock, neospiramycin is produced. The total amount of spiramycin and neospiramycin detected by absorptiometry is taken as the residual quantity in livestock.

■Analysis Conditions

Column	: Shim-pack VP- ODS(150mmL. × 4.6mm I.D.)
Mobile Phase	: 0.5M (Sodium)Phosphate Buffer(pH=2.5)/ Acetonitrile=3/1(v/v)
Flow Rate	: 0.5mL/min
Temperature	: 40˚C
Detection	: SPD- M10A*VP* at 235nm

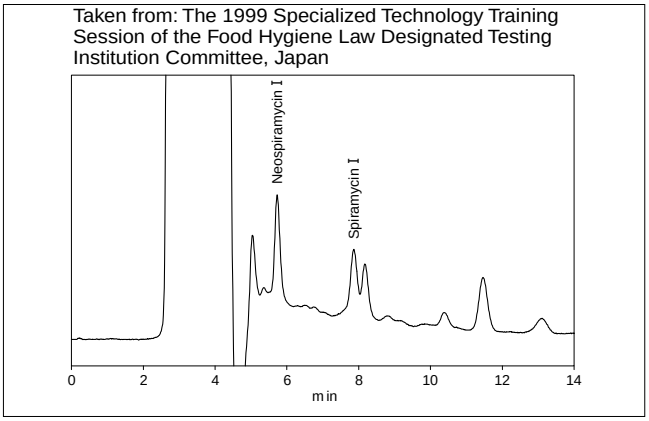


Fig. 2.6.1 Bovine Liver Extract

2.7 Simultaneous Analysis of Synthetic Antibacterial Agents (1) - LC

■Explanation

HPLC is recognized as the best method for analysis of food-residual (especially fish and meat) antibacterial agents and antibiotics.

References

Hamada, Murakita; Shimadzu Review, 52 (2), 107 (1995)
Murayama, Uchiyama, Saito, Food Hygiene Journal, 32, 155 (1991)
Milk Hygiene Volume 79, April 1993 (from the former Ministry of Health, Labour and Welfare)

■Pretreatment

Fig. 2.7.2 shows the method recommended by the former Ministry of Health, Labour and Welfare.

■Analytical Conditions

Column : STR ODS-Ⅱ (150mmL. × 4.6mm I.D.)
Mobile Phase : A : Water/Acetic Acid=100/0.3 (v/v) (containing NaClO4)
B : Acetonitrile/ Water/ Acetic Acid =90/10/0.3(v/v/v) (containing NaClO4)
A/B Gradient elution
Flow Rate : 2.0mL/min
Temperature : 40˚C
Detection : SPD-M10A$_{VP}$ at 275nm

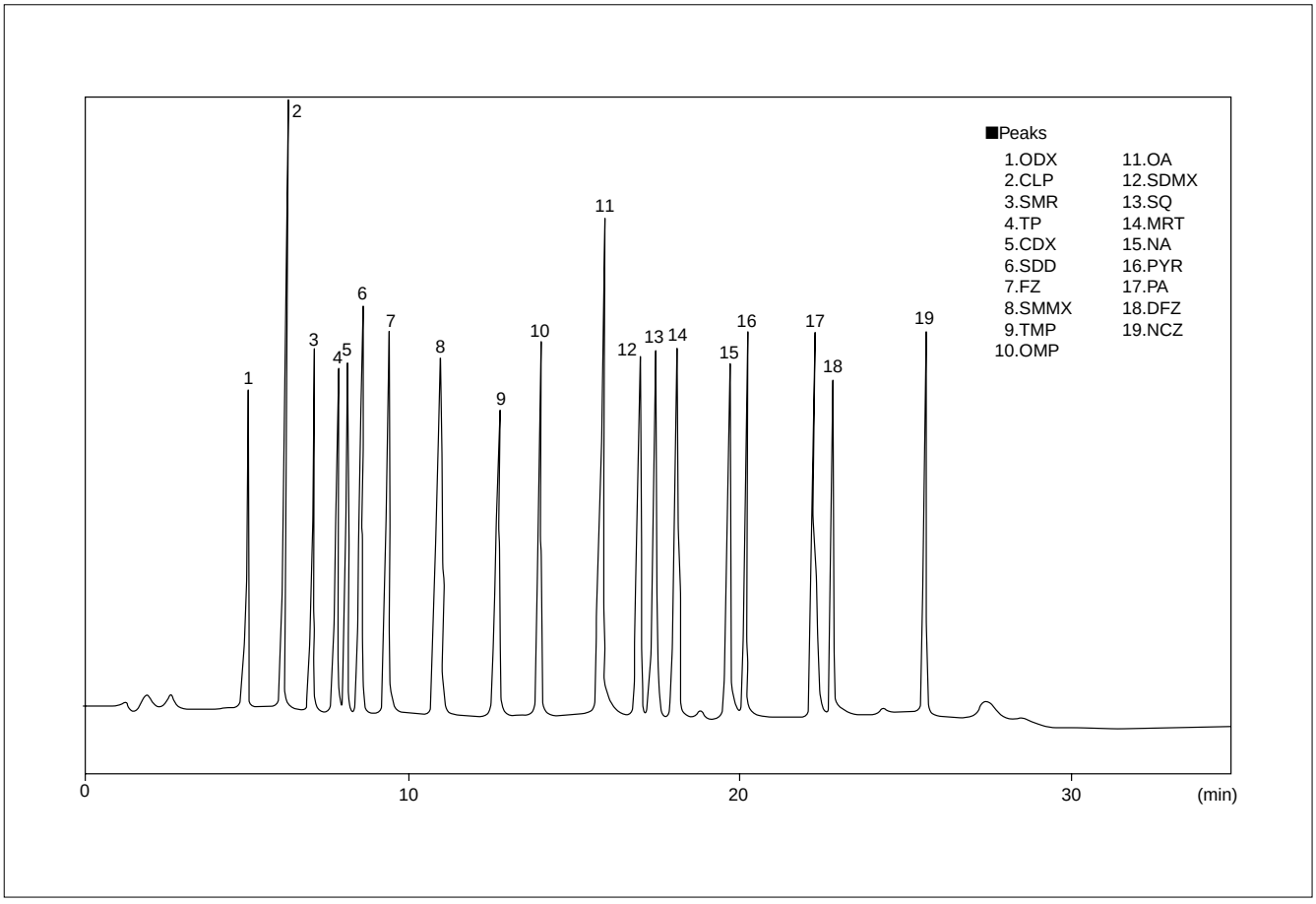


Fig. 2.7.1 Simultaneous analysis example for 19 synthetic antibacterial agent components

2.7 Simultaneous Analysis of Synthetic Antibacterial Agents (2) - LC

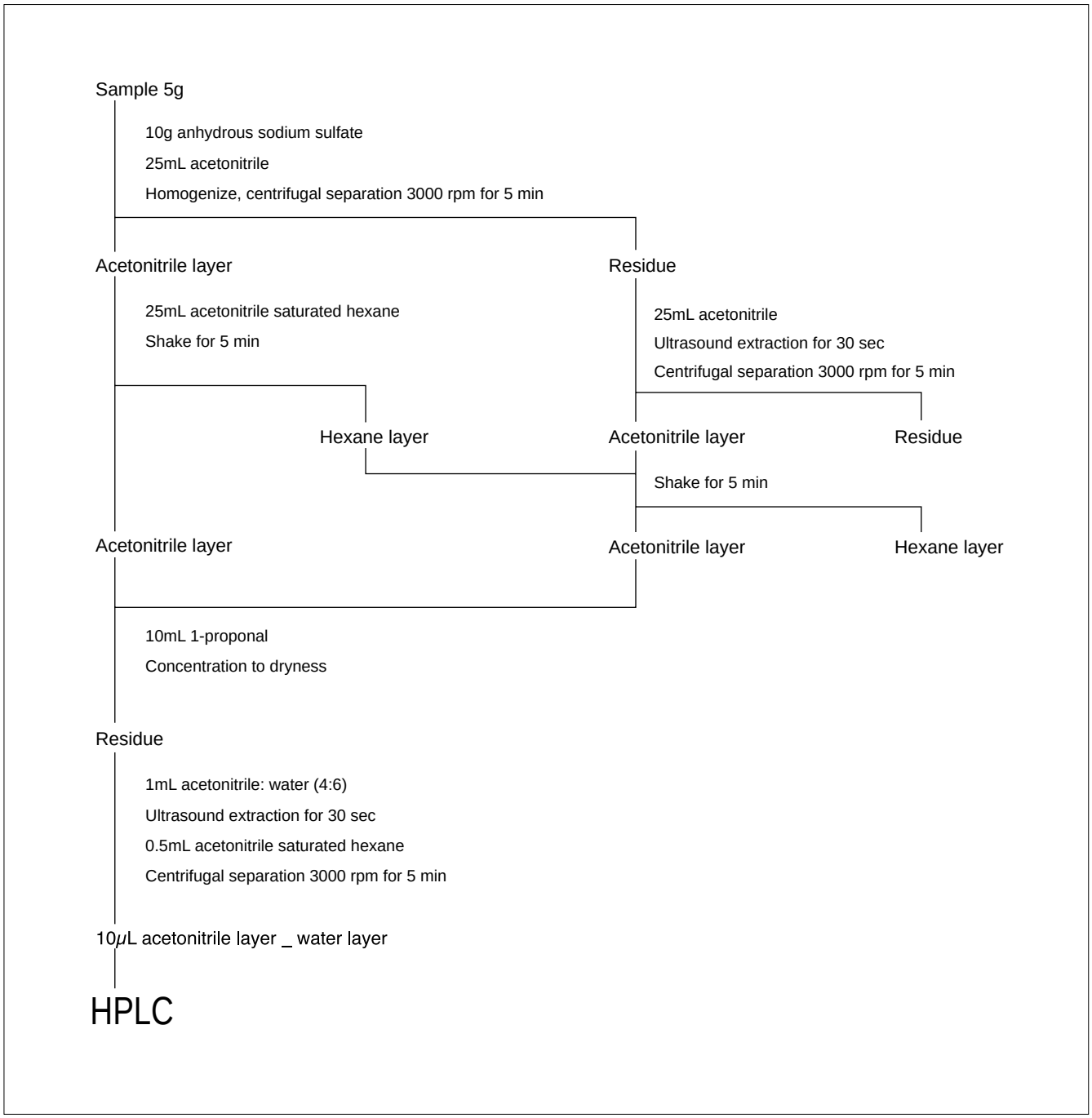


Fig. 2.7.2 Pretreatment flowchart for simultaneous analysis of 19 synthetic antibacterial agent components

2.8 Analysis of Sulfamethazine in Pork - LC

■Explanation

Fig. 2.8.2 shows chromatogram of sulfamethazine standard solution (200ppb), 20uL injection. Fig. 2.8.3 shows analysis of shop-sold pork with sulfamethazine 150ppb added.

■Pretreatment

See flowchart in Fig. 2.8.1.

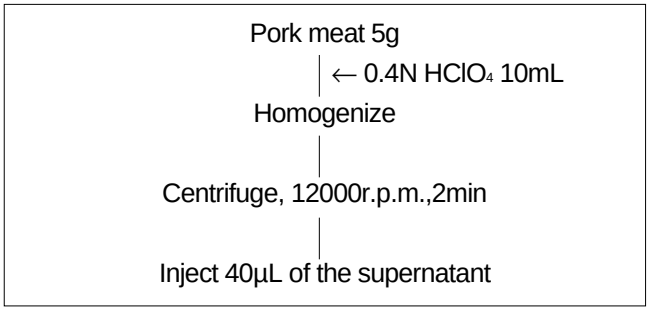


Fig. 2.8.1 Pretreatment

■Analytical conditions

Column : Shim-pack CLC-ODS(150mmL. × 6.0mm I.D.)
Mobile Phase : A: 10mM Sodium Phosphate Buffer(pH2.6)
B: Acetonitrile
A/B= 85/15 (v/v)
Flow Rate : 1.0 mL/min
Temperature : 40˚C
Detection : UV (250nm)

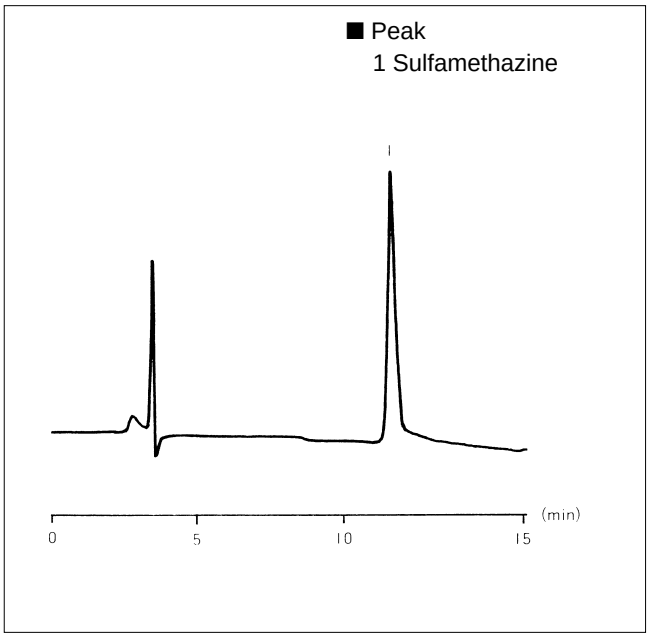


Fig. 2.8.2 Analysis of sulfamethazine standard

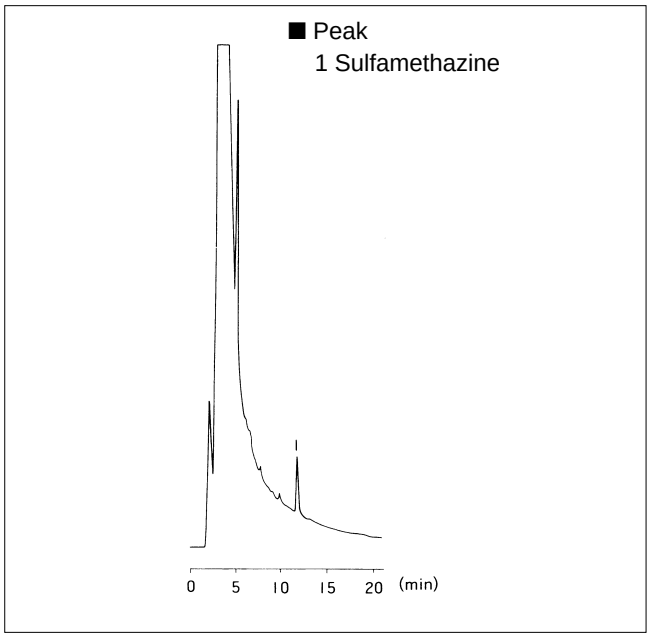


Fig. 2.8.3 Analysis of Sulfamethazine in Pork

2.9 Analysis of New Type Quinolone Antibacterial Agents in Poultry - LC

■Explanation

In Japan, some of the standards for food products and additives were revised in accordance with Notification No. 369 issued by the Ministry of Health, Labour and Welfare on 26 November 2003. New standards and test methods for the residual amounts of sarafloxacin and danofloxacin in meat were established, and LC-fluorescence is now used for qualitative and quantitative testing. In the example presented here, LC-fluorescence is used in the analysis of new type quinolone antibacterial agents (sarafloxacin and danofloxacin).

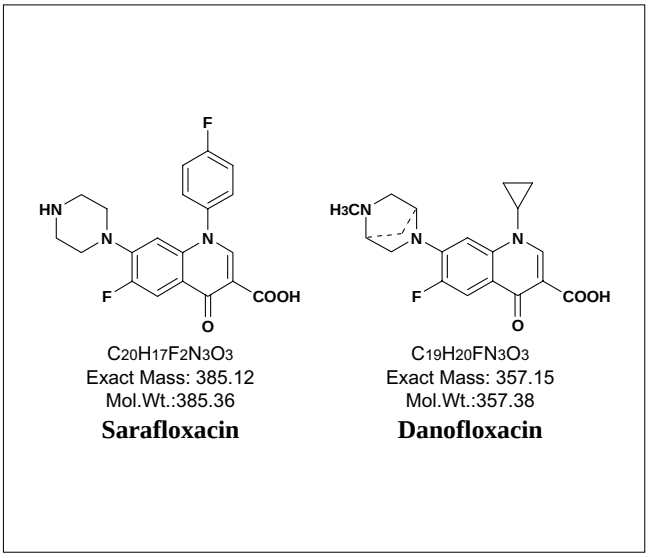


Fig. 2.9.1 Structure of sarafloxacin and danofloxacin

■Analysis Conditions

Column	: Shim-pack VP-ODS(150mmL. × 4.6mm I.D.)
Mobile Phase	: A: 0.05%TFA-Water
	B: Acetonitrile
	A/B= 4/1 (v/v)
Flow Rate	: 0.8mL/min
Temperature	: 40˚C
Sample Store Temp.	: 5˚C
Detection	: RF-10AXL (Ex:280nm Em:460nm)

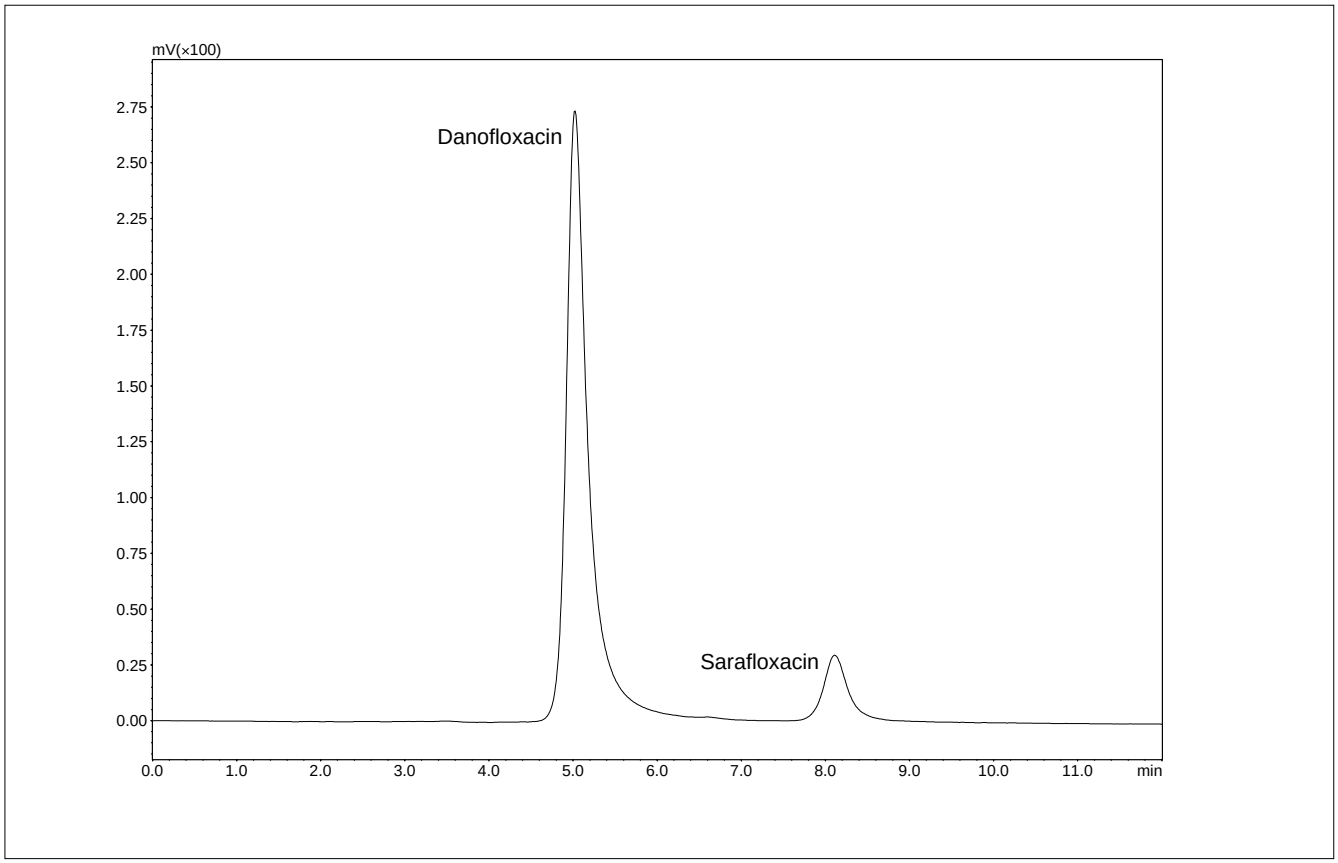


Fig. 2.9.2 Analysis of standard samples (50 ppb for each constituent, 10 µL injected)

2.10 Analysis of Synthetic Antibacterial Agents (1) - LC/MS

■Explanation

Synthetic anti-bacterial drugs are widely used as feed additives and pharmaceuticals for animals to improve production of livestock and marine products. However, in recent years, fears have been expressed about the shift to livestock and marine products produced with such drugs and the effects that such drug residues might have on humans. Liquid chromatography is used in analysis work on these synthetic anti-bacterial drugs. There are numerous target compounds in such analysis, and these need to be individually separated or separated into sample matrixes, while, moreover, the target compounds themselves also are difficult to identify. Consequently, there is a need for an analysis method with superior sensitivity that provides greater selectivity and abundant qualitative data.

Here, a LC-PDA-MS system with LCMS-QP8000α (LabSolutions S/W) comprising a photodiode array detector was used to analyze 11 products including thiamphenicol (TPC), sulfadimidine (SDD), sulfamonomethoxine (SMM), sulfadimethoxine (SDM) and oxolinic acid (OXA) that are greatly used in the production of synthetic anti-bacterial drugs designated as feed additives and animal pharmaceuticals. Fig. 2.10.1 and Fig. 2.10.2 show data acquired from qualitative information (MS and UV spectrums) using SCAN measuring, while Fig. 2.10.3 shows the results of a standard additive test for pig liver extract. The pig liver extract fluid was prepared in adherence with the "acetonitrile-hexane distribution method" laid down in the general analysis method for synthetic anti-bacterial drugs according to the official method (Ministry of Health, Labour and Welfare, Veterinary Sanitation Division Notification No. 78).

■Analytical Conditions

Model	: Shimadzu LCMS-QP8000α
Column	: Shim-pack VP-ODS (150 mmL. × 2.0 mm I.D.)
Mobile Phase A	: Water containing 0.3% Acetic Acid
Mobile Phase B	: 60% Acetonitrile-Water containing 0.3% Acetic Acid
Time Program	: 0%B(0 min)→70%B(30-32 min)
Flow Rate	: 0.2 mL/min
Injection Volume	: 50µL
Temperature	: 40°C
Ionization Mode	: ESI (+)
Neburaizing Gas Flow Rate	: 4.5 L/min
Applied Voltage	: 4.5kV CDL Temp. : 230 °C
CDL Voltage	: -30 V DEFs Voltage : + 40 V
Scan Range for SCAN Analysis	: m/z 100 - 400 (0.8 sec/scan)
Monitor Ions for SIM Analysis	: m/z 291, 265, 356, 358, 249, 251, 279, 226, 281, 361, 311, 301(0.8 sec/12 chs)

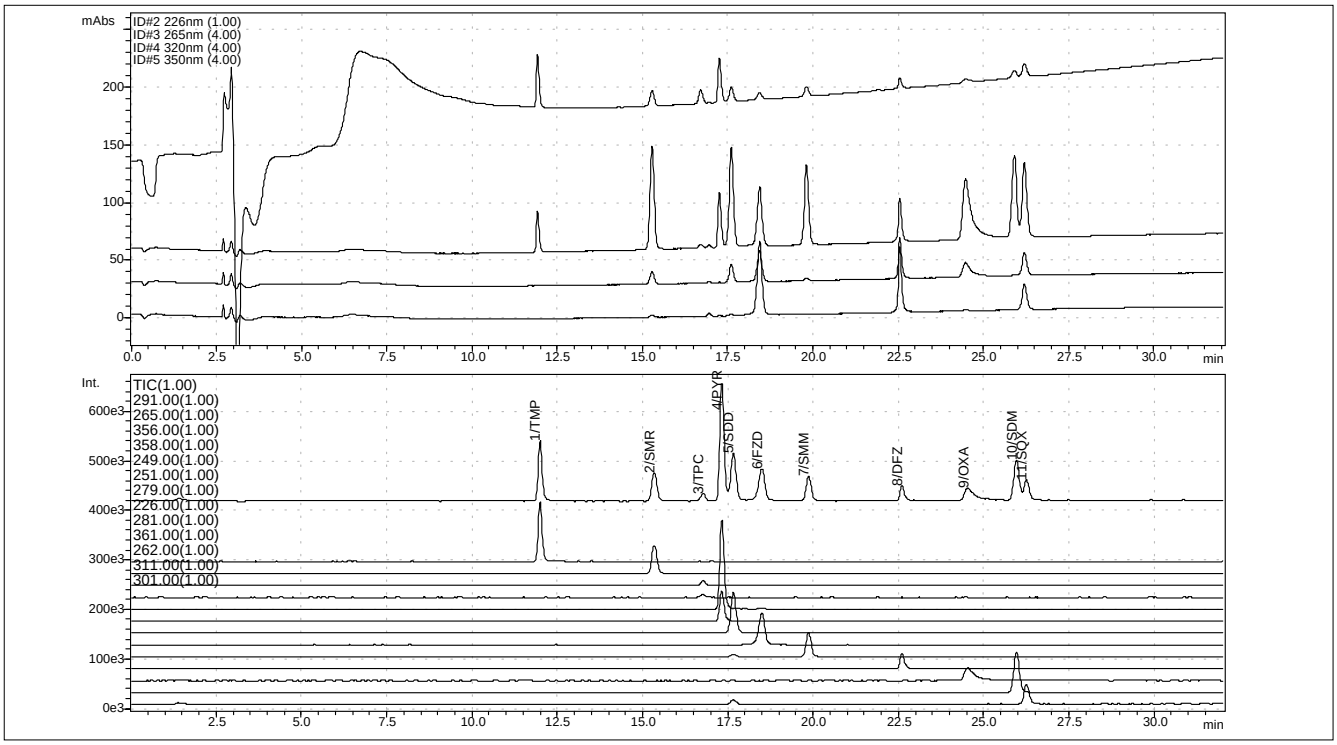


Fig. 2.10.1 Chromatogram of synthetic anti-bacterial standard(400ppb)

2.10 Analysis of Synthetic Antibacterial Agents (2) - LC/MS

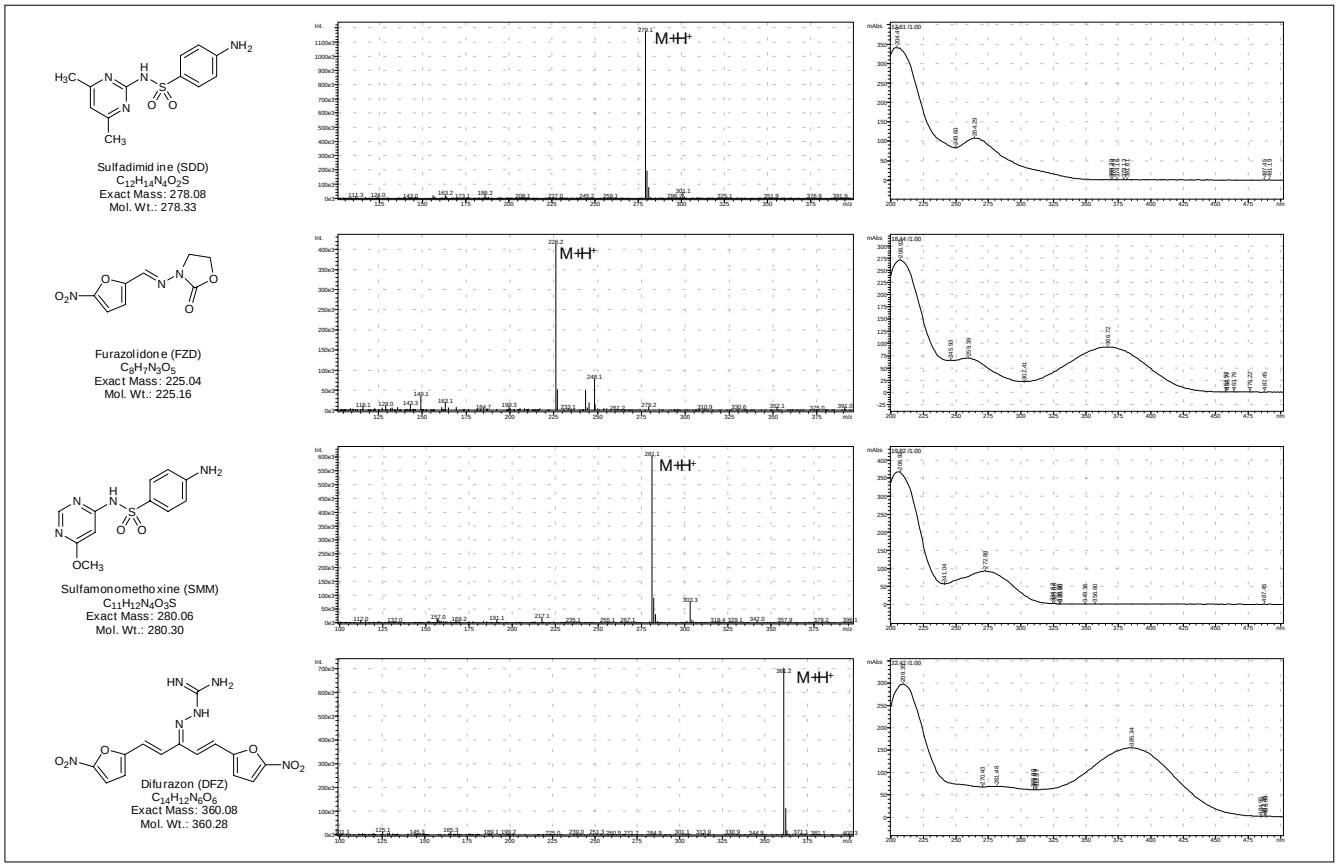


Fig. 2.10.2 Qualitative information (MS, UV) on synthetic anti-bacterial standards

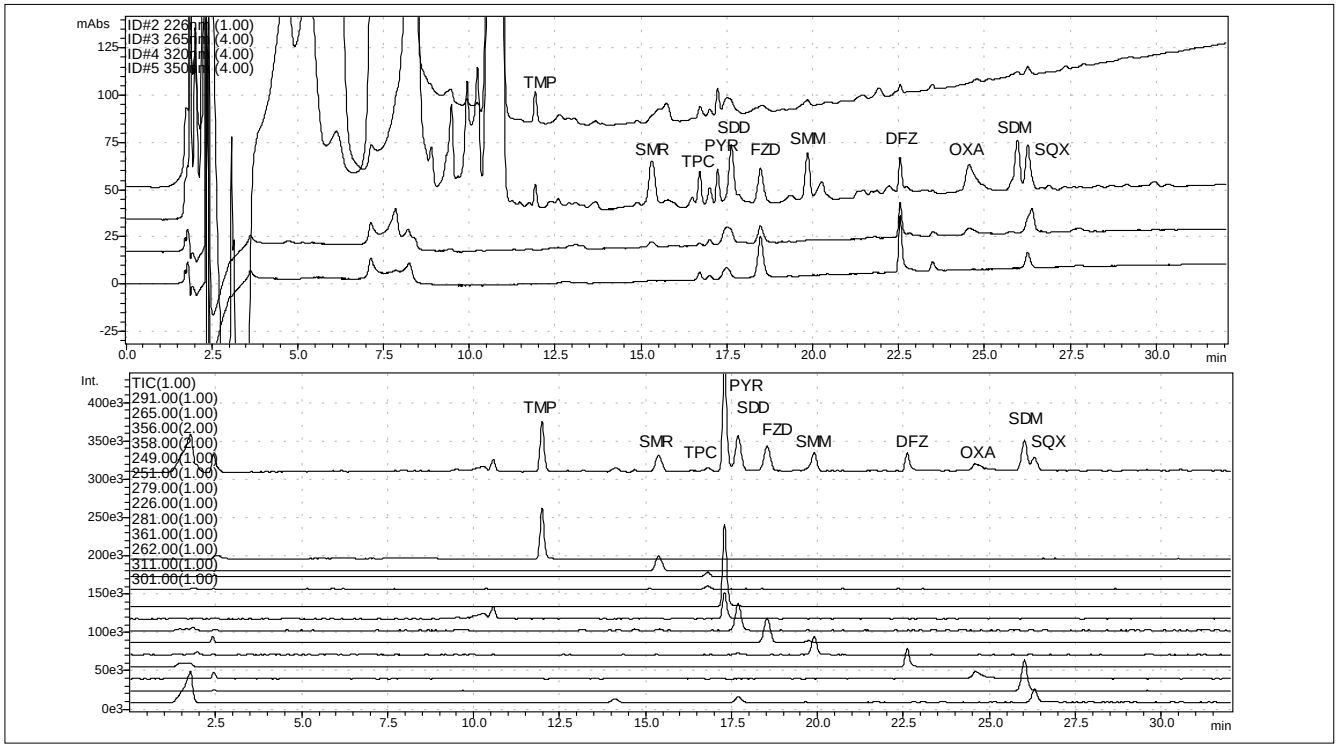


Fig. 2.10.3 Analysis results for pig liver extract liquid standard additive (200ppb)

2.11 Analysis of New Type Quinolone Antibacterial Agents in Poultry - LC/MS

■Explanation

In Japan, some of the standards for food products and additives were revised in accordance with Notification No. 369 issued by the Ministry of Health, Labour and Welfare on 26 November 2003. New standards and test methods for the residual amounts of sarafloxacin and danofloxacin in meat were established, and LC/MS is now used for confirmation tests. In the example presented here, LC/MS is used in the analysis of new type quinolone antibacterial agents (sarafloxacin and danofloxacin).

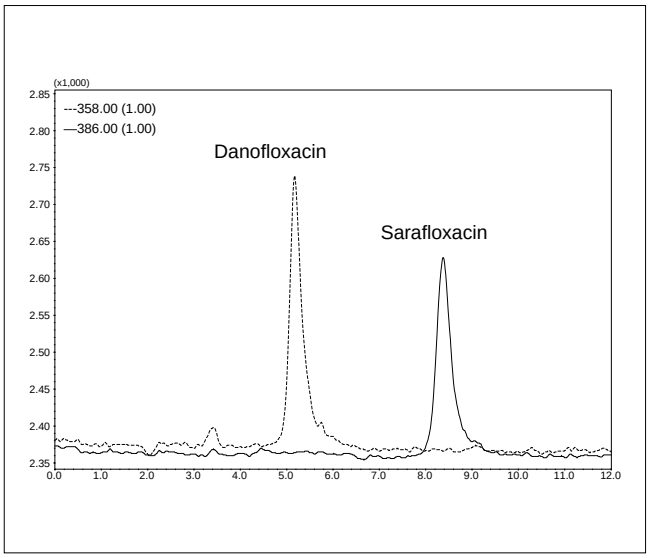


Fig. 2.11.1 Analysis of standard samples (50 ppb for each constituent, 50 µL injected)

■Analytical Conditions

Instruments	: LCMS-2010A
Column	: Shim-pack VP-ODS (150mmL. × 4.6mm I.D.)
Mobile Phase	: A: 0.05%TFA-Water
	B: Acetonitrile
	A/B= 4/1 (v/v)
Flow Rate	: 0.8mL/min
Temperature	: 40˚C
Sample Store Temp	: 5˚C
Ionization Mode	: ESI-Positive
Applied Voltage	: 4.5kV
Nebulizer Gas Flow	: 1.5L/min.
Drying Gas Pressure	: 0.2MPa
CDL Temp.	: 200˚C
Heat Block Temp.	: 200˚C
CDL Voltage	: S-Mode
Q-array Voltage	: S-Mode
Selected Ion Mass Number	: m/z 358.00 $(M+H)^+$ for Danofloxacin
	m/z 386.00 $(M+H)^+$ for Sarafloxacin

■Confirming Addition of Regulated-Level Concentration

Although unwanted peaks are obtained at retention times different to that of danofloxacin, the regulated-level concentration can be easily detected.

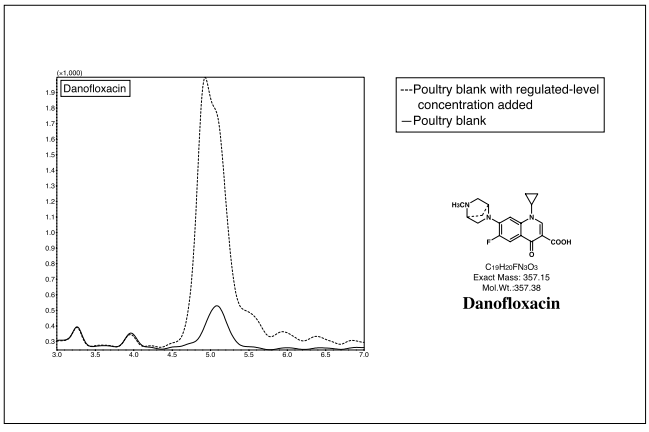


Fig. 2.11.2 Chromatogram for addition of regulated-level concentration (danofloxacin)

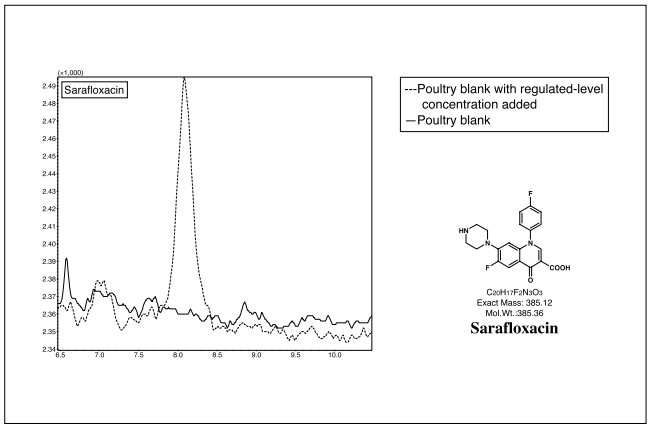


Fig. 2.11.3 Chromatogram for addition of regulated-level concentration (sarafloxacin)

2.12 Analysis of Aminoglycoside Antibiotics (1) - LC/MS

■Explanation

Some of the ministerial ordinances related to standards on the constituents of milk and dairy products were revised in accordance with Notification No. 170 issued by the Ministry of Health, Labour and Welfare on 26 November 2003. New standards and test methods (LC/MS) for the residual amounts of streptomycin and dihydrostreptomycin in milk were established. In the example presented here, the analysis of these two constituents and of gentamycins, spectinomycin, and neomycin, constituents for which LC/MS was already specified as the test method, is performed.

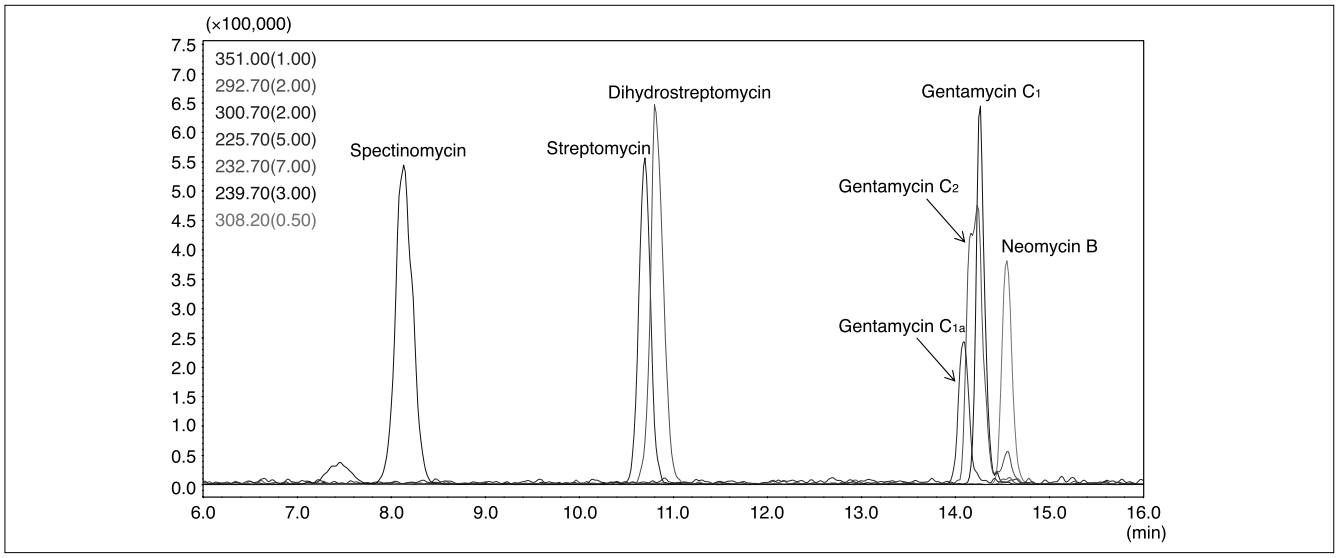


Fig. 2.12.1 ESI mass chromatogram for aminoglycoside antibiotics

■Analytical Conditions

Instruments	: Shimadzu LCMS-2010A
Column	: Shim-pack VP-ODS (150mmL. × 2.0mm I.D.)
Mobile Phase A	: 5mM Perfluorobutyric Acid (PFBA)-Water
Mobile Phase B	: Acetonitrile
Flow Rate	: 0.4mL/min
Gradient Program	: 10%B (0 min) → 40%B (15-20 min)
Column Temperature	: 40˚C
Injection Volume	: 10µL
Ionization Mode	: Positive ESI
Applied Voltage	: 4.5kV
Neburizing Gas Flow	: 1.5L/min
Drying Gas Pressure	: 0.1MPa
CDL Temperature	: 200˚C
Block Temperature	: 200˚C
CDL Voltage	: S-Mode
Selected Ion Mass Number	: m/z 351.0 for Spectinomycin m/z 300.7 for Streptmycin m/z 292.7 for Dihydrostreptmycin m/z 225.7 for Gentamycin C1a m/z 232.7 for Gentamycin C2 m/z 239.7 for Gentamycin C1 m/z 308.2 for Neomycin B

2.12 Analysis of Aminoglycoside Antibiotics (2) - LC/MS

■Samples with Standard of Regulated Concentration Added and Pork Blanks

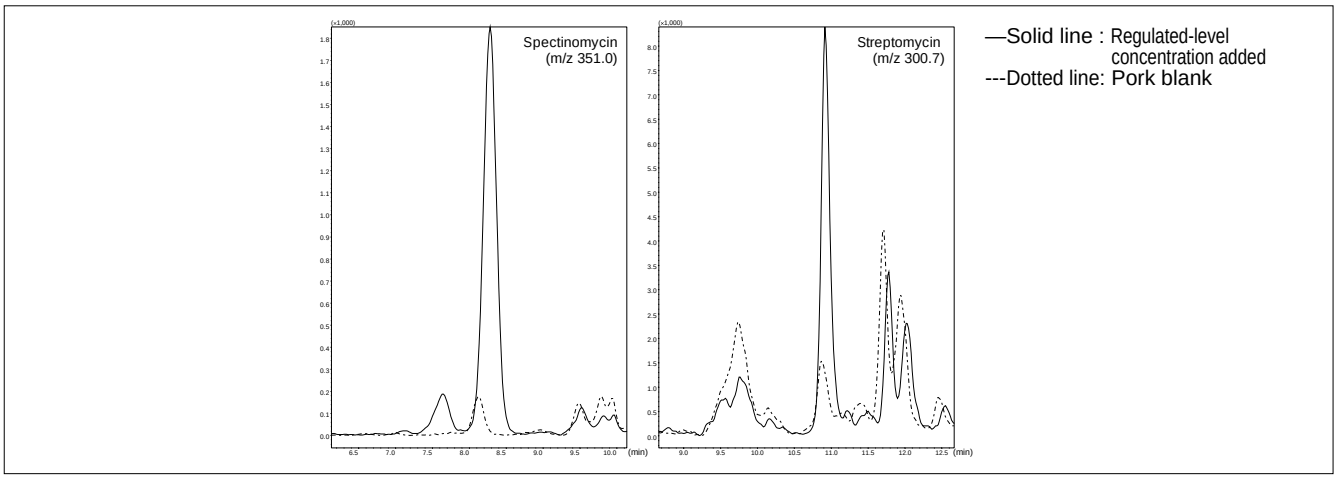


Fig. 2.12.2 Samples with standard of regulated-level concentration added and pork blanks (1)

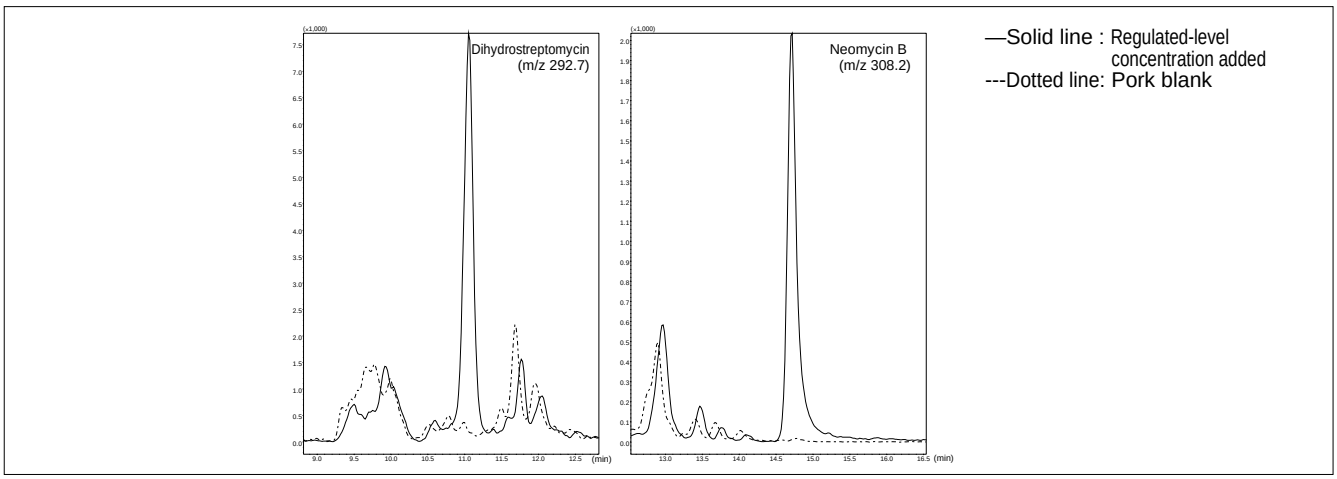


Fig. 2.12.3 Samples with standard of regulated-level concentration added and pork blanks (2)

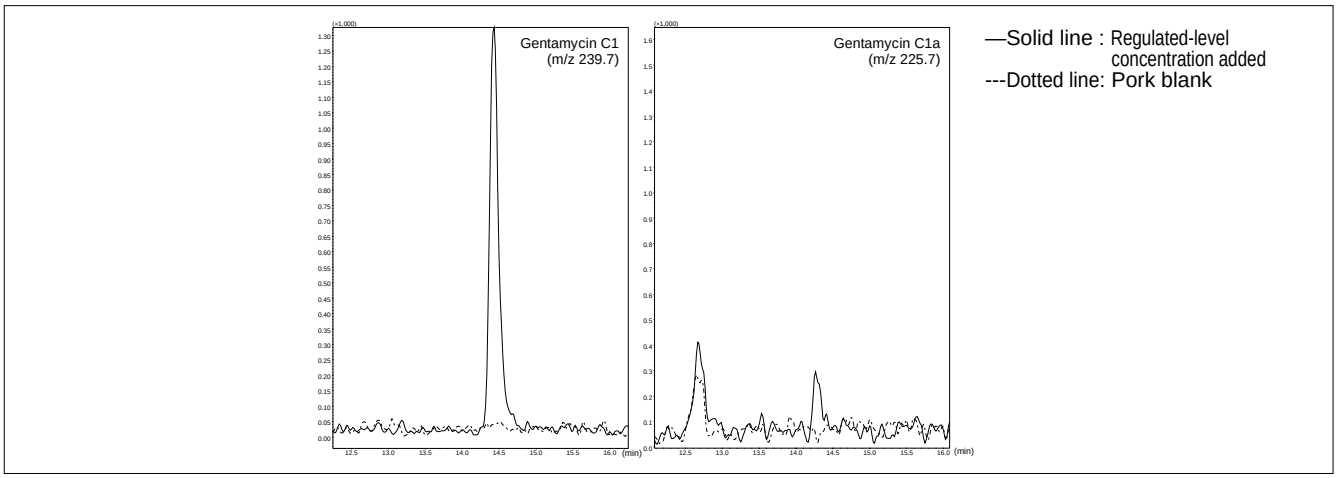


Fig. 2.12.4 Samples with standard of regulated-level concentration added and pork blanks (3)

2.13 Analysis of Flubendazole - LC

■Explanation

Fig. 2.13.1 shows the analysis results of a 1 ppm standard flubendazole solution. Fig. 2.13.2 shows the spectrum obtained using a photodiode array UV-Vis spectrophotometer detector.

Analytical conditions conform to the Japanese Ministry of Health, Labour and Welfare, Official Gazette Issue No. 245 (December 26, 1995).

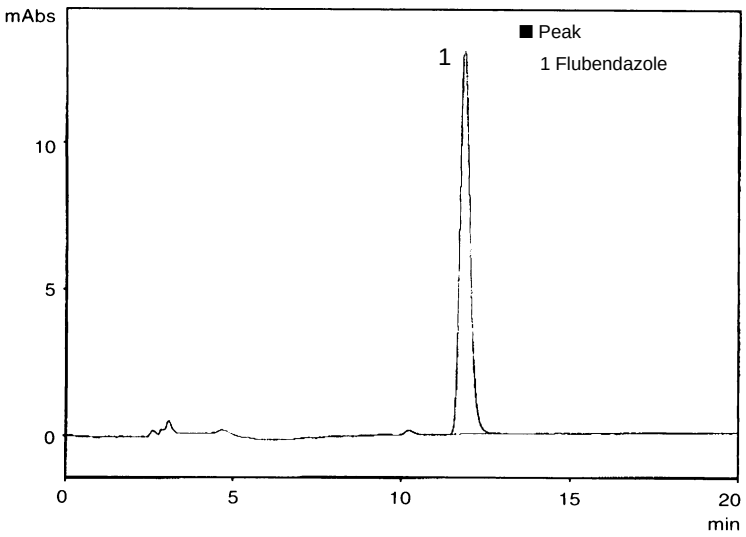


Fig. 2.13.1 Chromatogram of a Standard Flubendazole Sample (1 ppm)

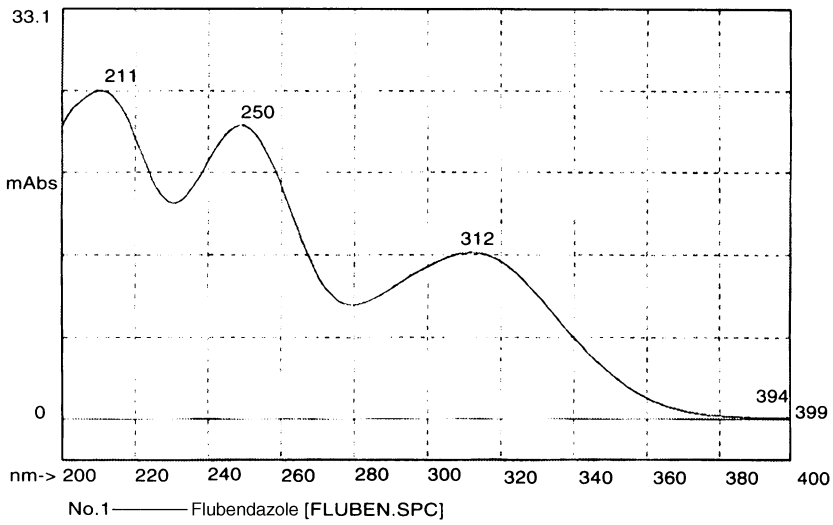


Fig. 2.13.2 Spectrum of a Standard Flubendazole Sample

2.14 Analysis of Closantel - LC

■Explanation

Smple was extracted from shop-sold pig liver using the official gazette method and closantel was added to make a solution of 1.0ppm for analysis. Fig. 2.14.3 shows comparison of spectra of closantel standard and pork extract sample.

References

Official Gazette extra No. 245 (December 26, 1995)

■Pretreatment

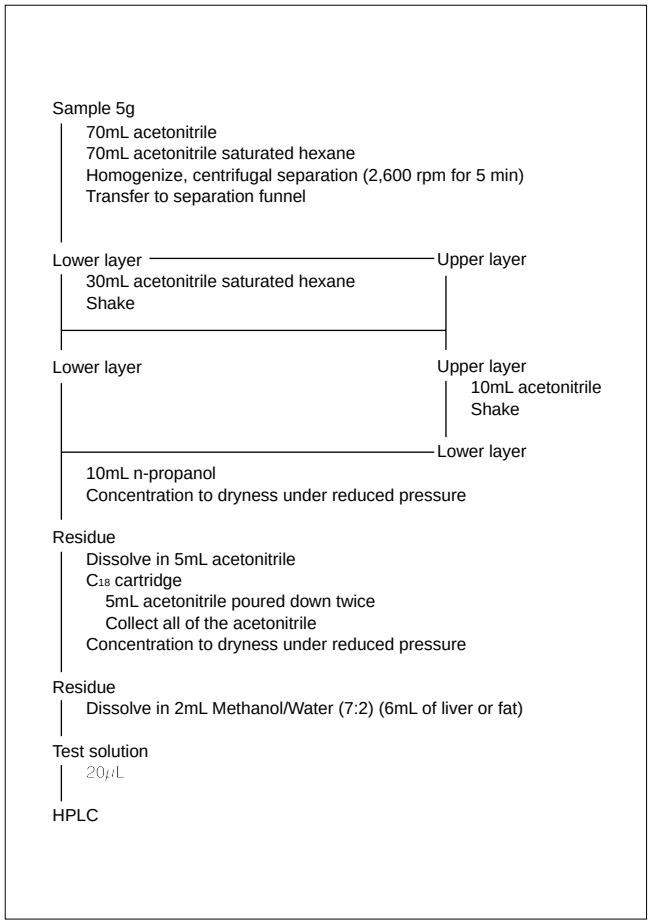


Fig. 2.14.1 Pretreatment flowchart for closantel

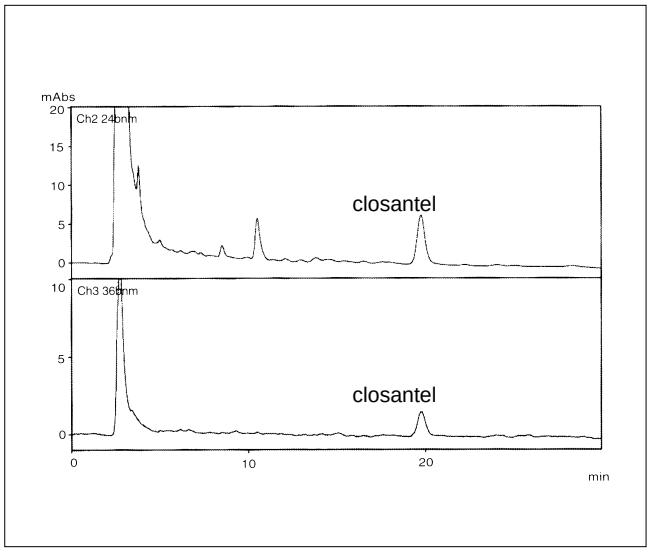


Fig. 2.14.2 Shop-sold pig liver extract (closantel 1.0ppm added)

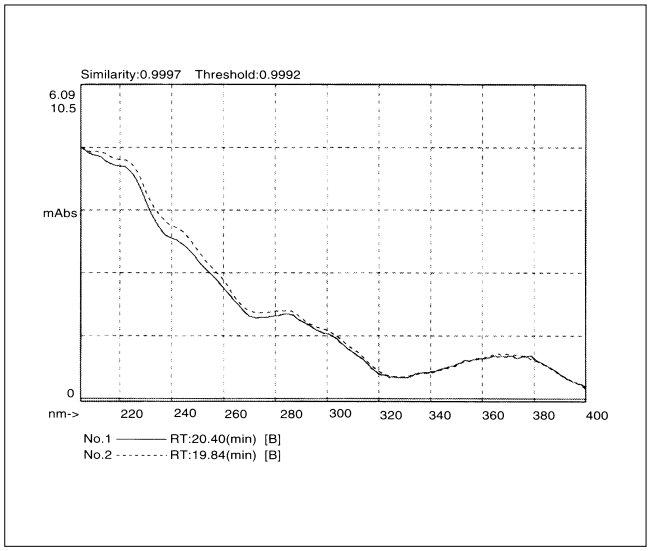


Fig. 2.14.3 Comparison of spectra of closantel standard and pork extract

2.15 Analysis of Isometamidium - LC

■Analysis of Isometamidium

An octyl silylated silica gel column was used as the analytical column and the substance was detected by absorptiometry. Since isometamidium in the solution state is less stable than other veterinary drugs, care must be taken during pretreatment.

■Analysis Conditions

Column : Shim-pack CLC-C8(M) (150mmL. × 4.6mm I.D.)
Mobile Phase : A: 30mM Citrate Buffer
containing 5mM Sodium 1-Heptanesulfonic Acid
B: Acetonitrile
A/B= 7/3(v/v)
Flow Rate : 1.2mL/min
Temperature : 40˚C
Detection : SPD-M10A*VP* at 380nm

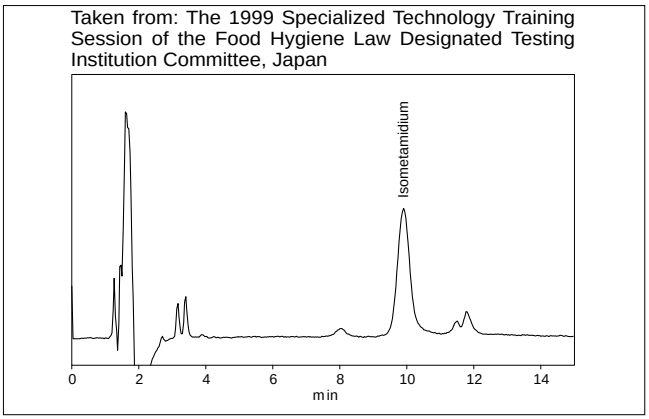


Fig. 2.15.1 Bovine Liver Extract
(0.5 ppm isometamidium added)

2.16 Analysis of Triclabendazole - LC

■Analysis of Triclabendazole

When triclabendazole is metabolized within the body, the 2-methylthio group is converted into methylsulfinyl and additionally into methylsulfonyl groups. In the analysis of triclabendazole, triclabendazole and these metabolites oxidized with hydrogen peroxide are detected by absorptiometry. The oxidation is performed using a pre-column reaction.

■Analysis Conditions

Column : Shim-pack VP-ODS (150mmL. × 4.6mm I.D.)
Mobile Phase : A: 25mM Sodium Dihydrogen Phosphate Buffer
B: Acetonitrile
A/B= 1/1(v/v)
Flow Rate : 1.0mL/min
Temperature : 40˚C
Detection : UV 295nm (Precolumn Derivatization Method)

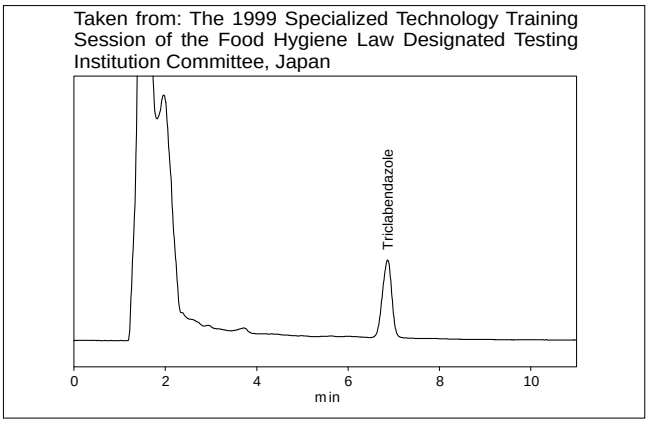


Fig. 2.16.1 Bovine Liver Extract
(0.3 ppm triclabendazole added)

2.17 Analysis of Ivermectin and Moxidectin - LC

■Analysis of Ivermectin and Moxidectin

Ivermectin and moxidectin were detected by florescence after the pre-column reaction with a fluorescence derivatization reagent.

■Analysis Conditions

Column : Shim-pack VP-ODS (150mmL. × 4.6mm I.D.)
Mobile Phase : Water/ Methanol = 3/ 97(v/v)
Flow Rate : 1.0mL/min
Temperature : 40˚C
Detection : Fluorescence Ex : 360nm, Em : 460nm
(Precolumn Derivatization Method)

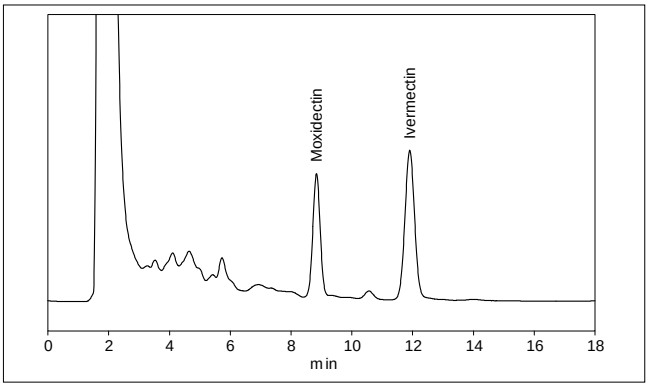


Fig. 2.17.1 Bovine Muscle Extract
(0.04 ppm ivermectin and 0.02 ppm moxidectin added)

2.18 Analysis of Ivermectin like Compounds (antiparasitic agents) (1) - LC/MS

■Explanation

Antiparasitic agents are veterinary medicines for animals used to exterminate parasites in livestock and are differentiated from medicines for humans. However, residual values for each component in food have been prescribed due to concerns about their negative effects on human health because humans indirectly consume minute doses of these agents when livestock is used for human consumption and Official regulations prescribes derivatization and HPLC analysis for ivermectin, a well-known antiparasitic agent.

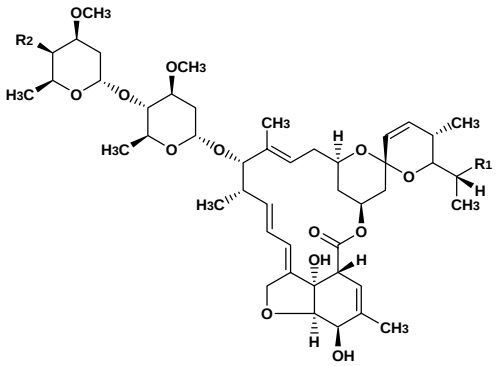
In practice this is a difficult analysis due to the effects of many obstructing components.One of these ivermectin-like compounds, emamectin, is sprayed on crops as an insecticide and is similarly prescribed in crop residue analysis laws of the Environment Agency.

This report describes an example of the simultaneous analysis of ivermectin-like compounds (Fig. 2.18.1).

Fig. 2.18.2 shows the total ion chromatogram analyzed in the SCAN mode.

Fig. 2.18.3 shows the mass spectra of emamectin B1a, 8, 9-Z-emamectin B1a, abamectin B1a, and ivermectin. The mass spectra are displayed at m/z 700-1000, each viewed with the protonated molecule as a base peak.

Fig. 2.18.4 shows the calibration curves in the range from 40 to 1000 pg. In each case good linearity was confirmed with a correlation coefficient of at least 0.999.



1 : Emamectin B1a	R_1=-C_2H_5	R_2=-$NHCH_3$
2 : FA emamectin B1a	R_1=-C_2H_5	R_2=-NCOH
3 : DM emamectin B1a	R_1=-C_2H_5	R_2=-NH_2
4 : 8,9-z-emamectin B1a	R_1=-C_2H_5	R_2=-$NHCH_3$
5 : MFA emamectin B1a	R_1=-C_2H_5	R_2=-$N(CH_3)COH$
6 : Abamectin B1a	R_1=-C_2H_5	R_2=-CH_3
7 : Ivermectin	R_1=-C_2H_5	R_2=-OH

Fig. 2.18.1 Structures of Ivermectin like compounds

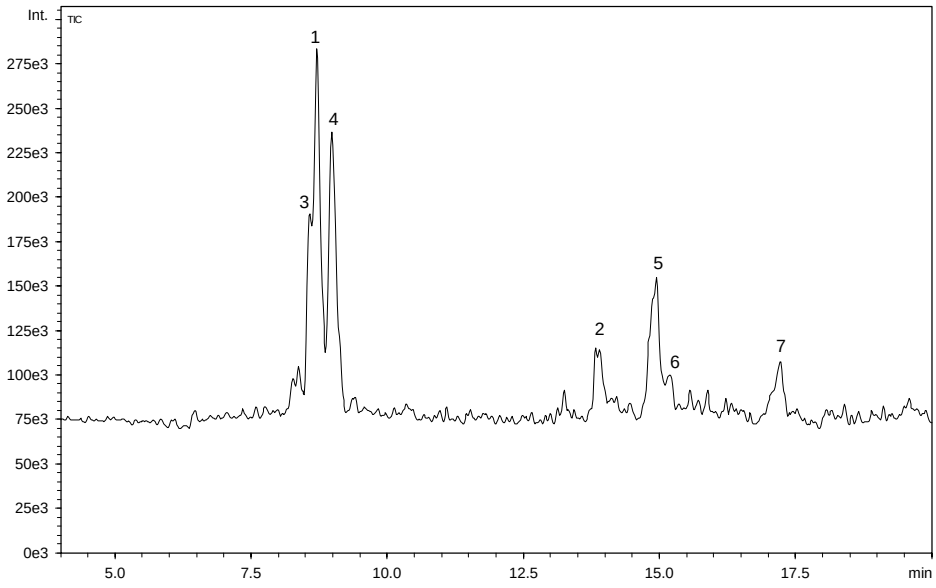


Fig. 2.18.2 Total ion chromatogram of ivermectin like compounds

2.18 Analysis of Ivermectin like Compounds (antiparasitic agents) (2) - LC/MS

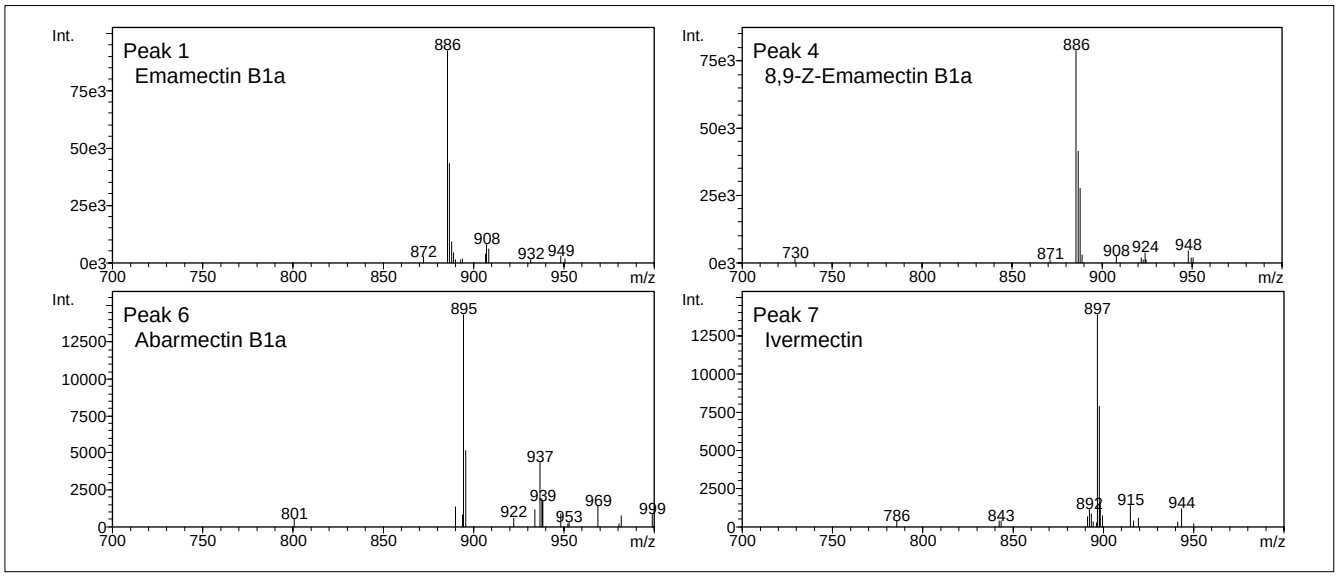


Fig. 2.18.3 Mass spectra of ivermectin like compounds

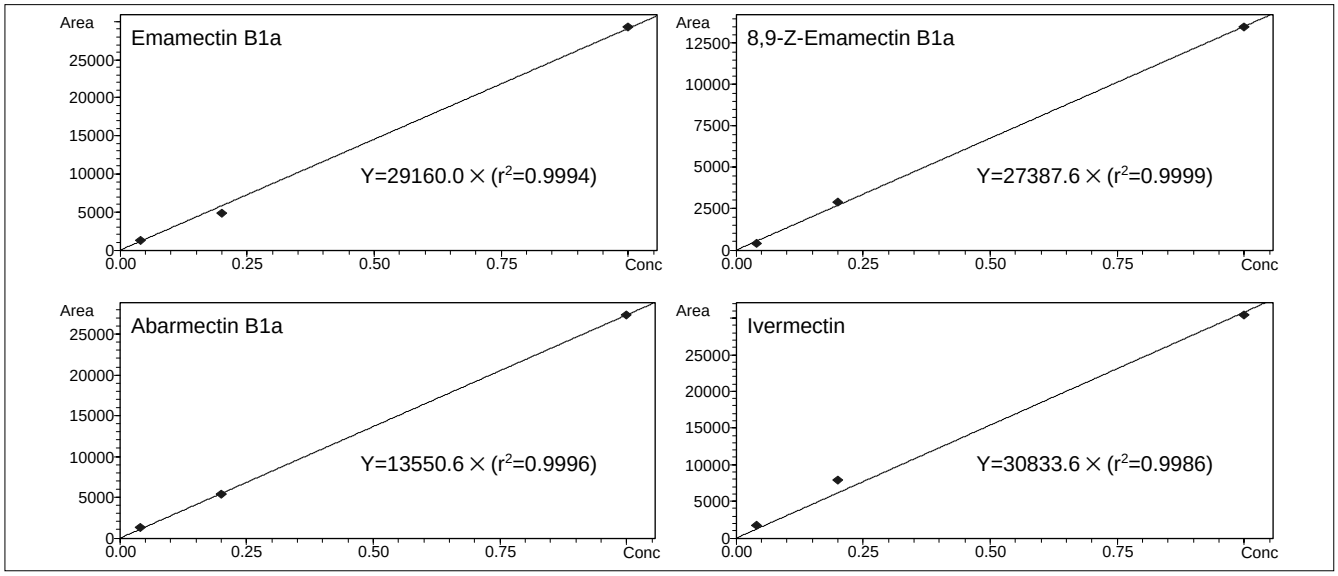


Fig. 2.18.4 Calibration curves of ivermectin like compounds

■Analysis Conditions

Column	: STR ODS-II (150 mmL. × 2.0 mm I.D.)
Mobile Phase A	: Water containing 0.2% Acetic Acid
B	: Acetonitrile containing 0.2% Acetic Acid
Gradient Program	: 30%B (0 min) → 100%B (10 - 20 min)
Flow Rate	: 0.2mL/min
Column Temperature	: 40˚C
Probe Voltage	: +4.5kV (ESI-Positive Mode)
Nebulizing Gas Flow	: 4.5L/min
CDL Voltage	: -60V
DEFs Voltage	: +49V
Scan Range	: m/z 300 - 1100

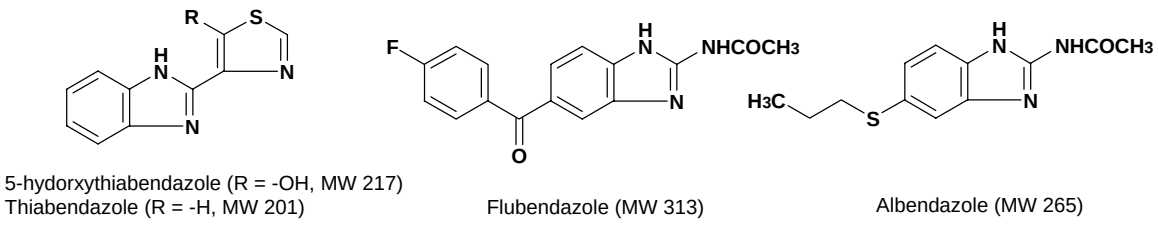
2.19 Analysis of Antiparasitic Agents (1) - LC/MS

Explanation

Veterinary pharmaceuticals including antibiotics and hormones are used to prevent disease in livestock, promote growth, and enhance the feed efficiency. Antiparasitic agents are also widely used to eliminate parasites from the alimentary canal. Residual standards are being established for antiparasitic agents as residual levels in meat present similar health problems to antibiotics and hormones.

The four components used for this test were 5-hydroxythiabendazole, thiabendazole, flubendazole, and albendazole. Their structures are shown in Fig. 2.19.1. A residual standard is set for each of these components in food. HPLC is prescribed for the analysis of these components but LC/MS permits analysis with extremely high selectivity and sensitivity.

Fig. 2.19.2 shows the LC/MS analysis results for the four antiparasitic agents. Each component could be positively identified using mass chromatography at the mass number of the protonated molecule of each component. Fig. 2.19.3 shows their mass spectra. Selected ion monitoring (SIM) permits highly sensitive analysis. Fig. 2.19.4 shows the calibration curves in the range from 10 to 1000 ppb. Each curve shows good linearity.



5-hydorxythiabendazole (R = -OH, MW 217)
Thiabendazole (R = -H, MW 201)

Flubendazole (MW 313)

Albendazole (MW 265)

Fig. 2.19.1 Structures of antiparasitic agents

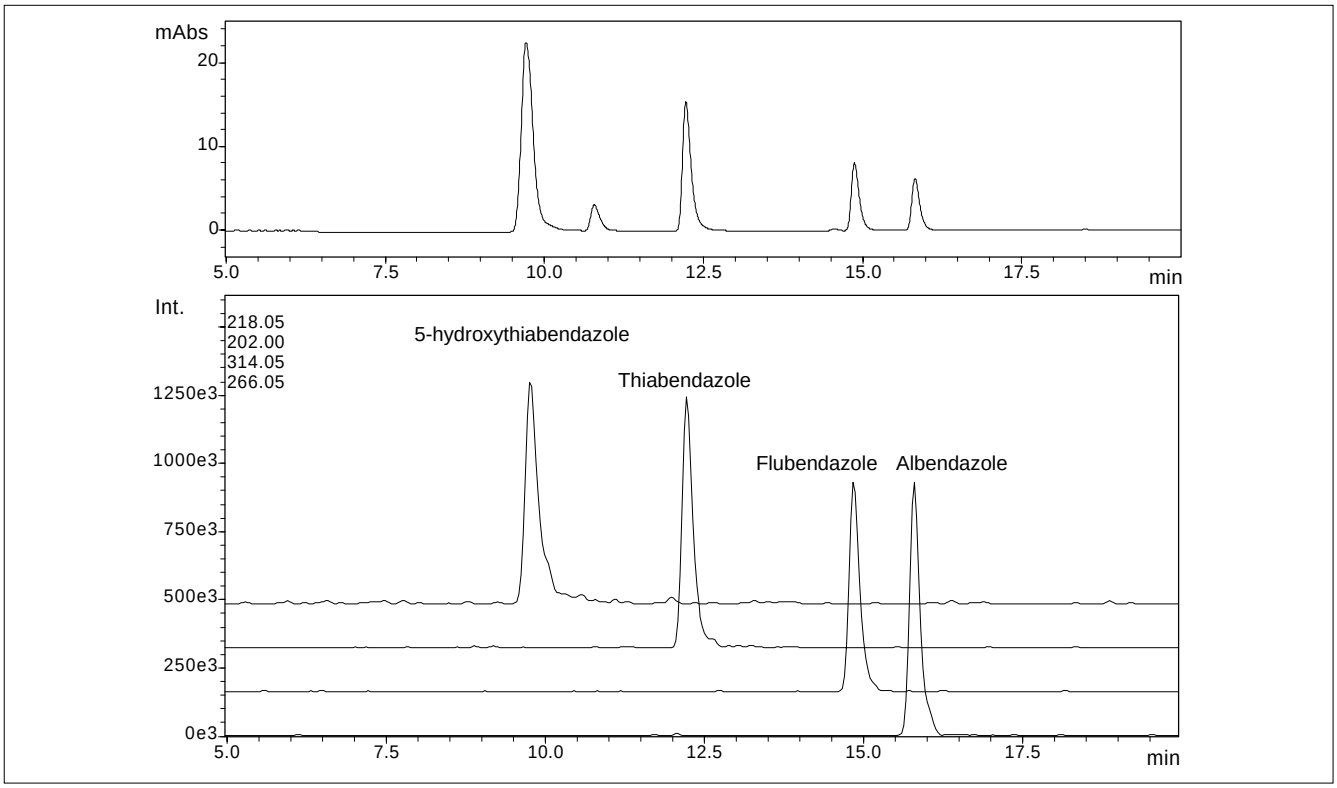


Fig. 2.19.2 UV absorption (305nm) and mass chromatograms of antiparasitic agents

2.19 Analysis of Antiparasitic Agents (2) - LC/MS

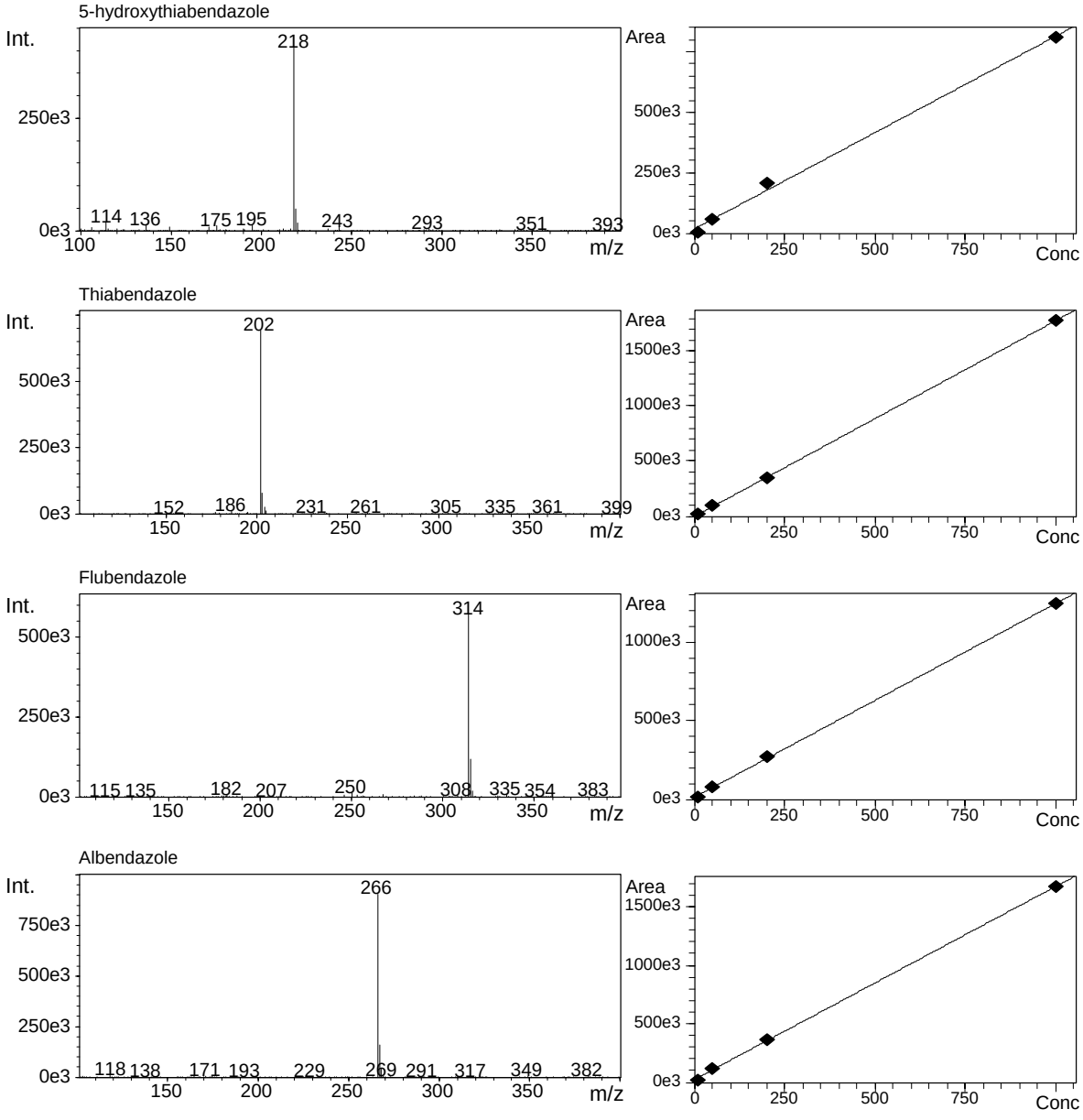


Fig. 2.19.3 Mass spectra of antiparasitic agents

Fig. 2.19.4 Calibration curves(10-1000ppb)

■Analysis Conditions

Column	: Inertsil ODS-2 (150 mmL. × 2.0 mm I.D.)
Mobile Phase A	: 5mM Acetic Acid-Ammonium Acetate Buffer
B	: Acetonitrile
Gradient Program	: 0%B (0min) → 100%B (20min)
Flow Rate	: 0.2mL/min
Column Temperature	: 40˚C
Probe Voltage	: +4.5kV (ESI-Positive Mode)
Nebulizing Gas Flow	: 4.5L/min
CDL Voltage	: -40 V (0 - 14min), -50 V (14.01 - 25min)
DEFs Voltage	: +40 V (0 - 14min), +45 V (14.01 - 25min)
Scan Range	: m/z 100 - 400

2.20 Analysis of Zeranol - LC

■Explanation

Fig. 2.20.1 shows the result of analyzing a 10 ppm standard zeranol solution in accordance with Japanese Ministry of Health, Labour and Welfare, Official Gazette Issue No. 245 (December 26, 1995). Fig. 2.20.2 shows the spectrum.

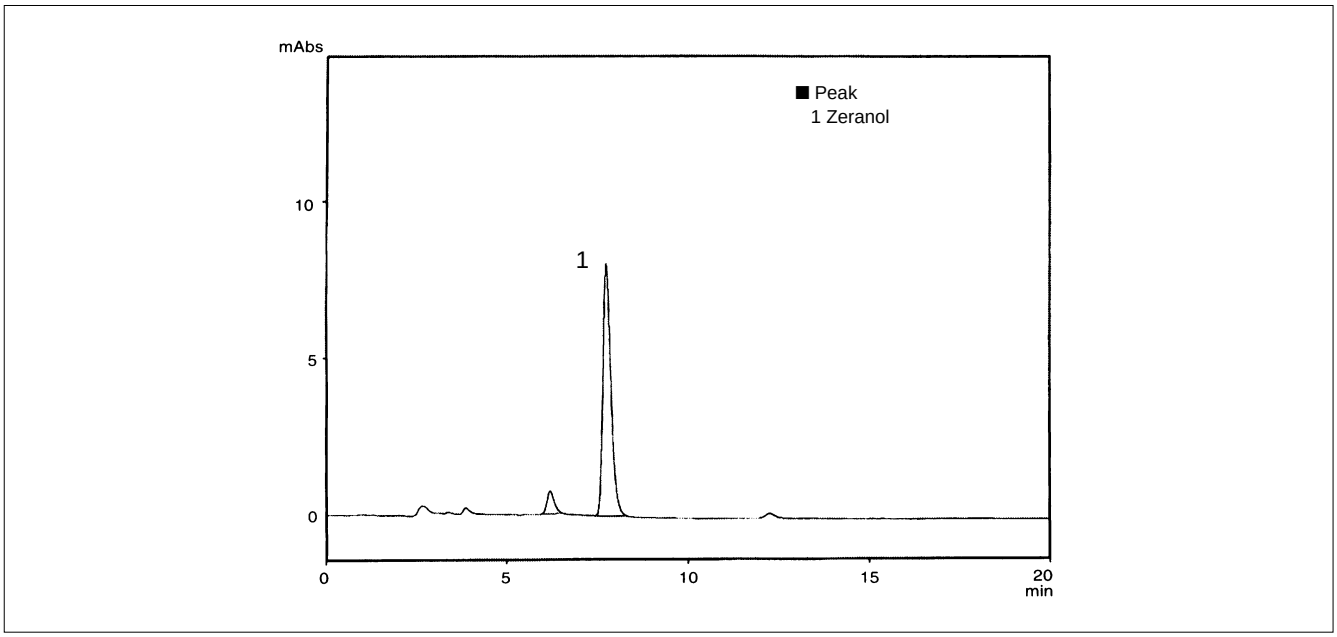


Fig. 2.20.1 Chromatogram of a Standard Zeranol Sample

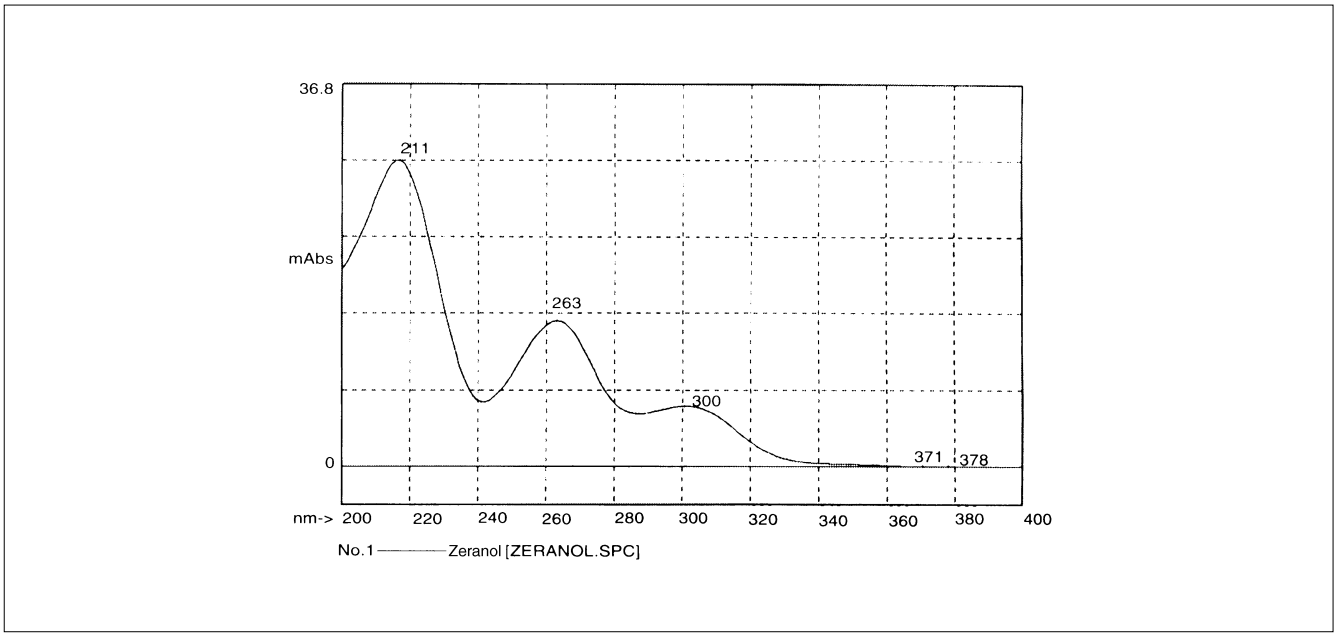


Fig. 2.20.2 Spectrum of a Standard Zeranol Sample

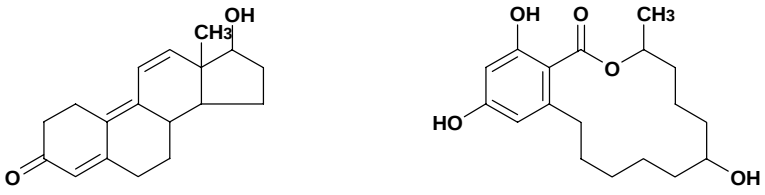
2.21 Analysis of Hormone Agents (1) - LC/MS

■Explanation

We consume fish and meat as part of our normal daily diets and healthy human life would be impossible without nutrition from such foodstuffs. Consequently not only a stable production and supply but safety of farm products, meat, and fish are demanded. Many agricultural chemicals and veterinary pharmaceuticals are currently used to increase productivity, but their residues in food are a problem.

This report describes an analysis example of hormones used for livestock. Hormones are used to promote growth in livestock and their safety is investigated in the same way as antibiotics and antiparasitics. The residual regulation values are established according to the level harmless to humans.

The structure of the hormones used in this analysis are shown in Fig. 2.21.1. Atmospheric-pressure chemical ionization (APCI) was used for the ionization in LC/MS. Figs. 2.21.2 and 2.21.3 show their mass chromatograms and mass spectra. α-and β-trenbolone exhibit a mass spectrum with the protonated molecule as the base peak, while zeranol was detected with the dehydrated ion of the protonated molecule as the base peak.



Trenbolone : $C_{18}H_{22}O_2$
Exact Mass : 270.16
Mol. Wt. : 270.37

Zeranol : $C_{18}H_{26}O_5$
Exact Mass : 322.18
Mol. Wt. : 322.40

Fig. 2.21.1 Structures of Trenbolone and Zeranol

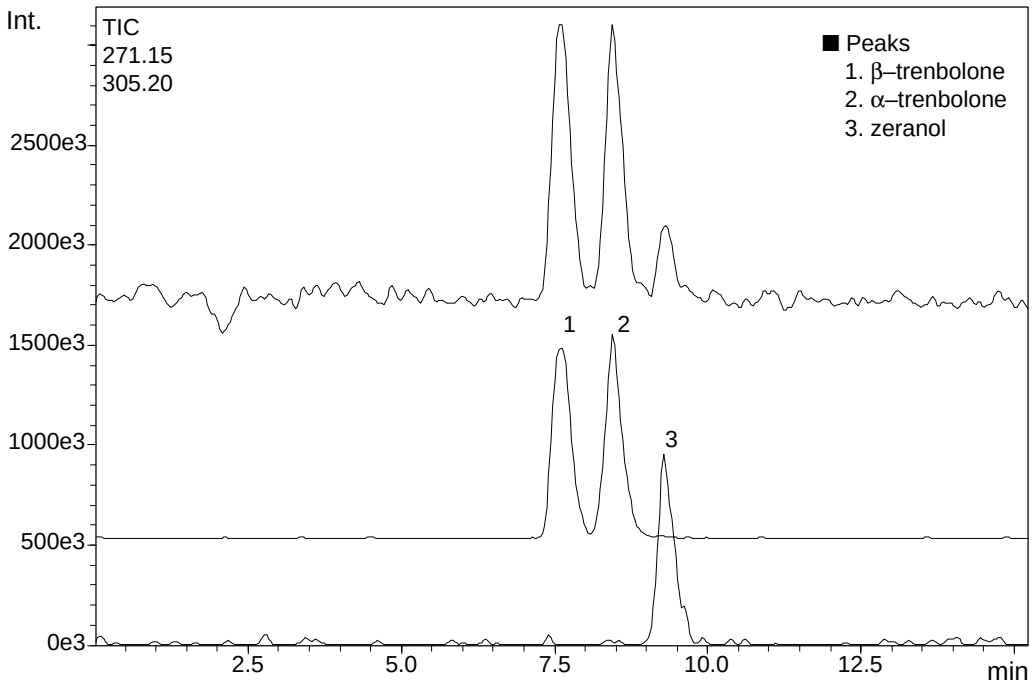


Fig. 2.21.2 Mass chromatograms of hormone agents

2.21 Analysis of Hormone Agents (2) - LC/MS

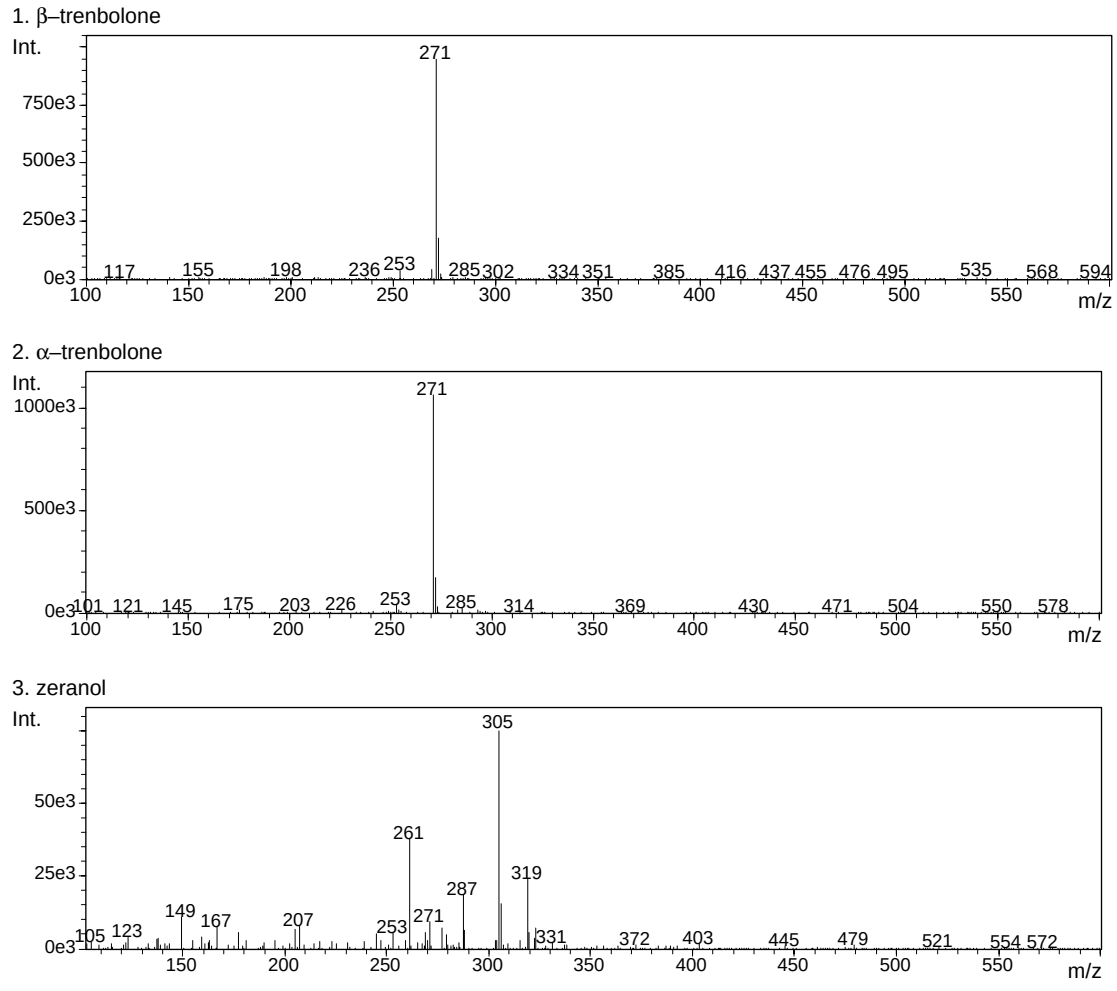


Fig. 2.21.3 Mass spectra of hormone agents

■Analytical conditions

Column	: STR ODS-II (150 mm L. × 2.0 mm I.D.)
Mobile Phase	: 60% Methanol-Water containing 0.3% Acetic Acid
Flow Rate	: 0.2mL/min
Column Temperature	: 40˚C
Probe Voltage	: +4.5kV (APCI-Positive Mode)
CDL Temperature	: 230˚C
Probe Temperature	: 400˚C
Nebulizing Gas Flow	: 2.5L/min
CDL Voltage	: -30V
DEFs Voltage	: +47V
Scan Range	: m/z 100-500

3.1 Analysis of p-Hydroxybenzoates in Soy Sauce - LC

■Explanation

LC is a great force in the analysis of preservatives used in food products. In particular, LC is useful for simultaneous analysis of such components. Here, an analysis example for p-hydroxybenzoates added to soy sauce will be introduced.

References

Shimadzu Application News No. L222 (C190-E032)

■Pretreatment

1. Add pure water to soy sauce until diluted by 10 fold.
2. Filter through membrane filter.
3. Inject 10µL of filtrate.

■Analytical Conditions

Column	: STR ODS-II (150mmL. × 4.6mm I.D.)
Mobile phase	: 10mM Sodium Phosphate Buffer (pH 2.6)/Methanol = 1/1 (v/v)
Temperature	: 40°C
Flow rate	: 1.5mL/min
Detection	: UV 270nm

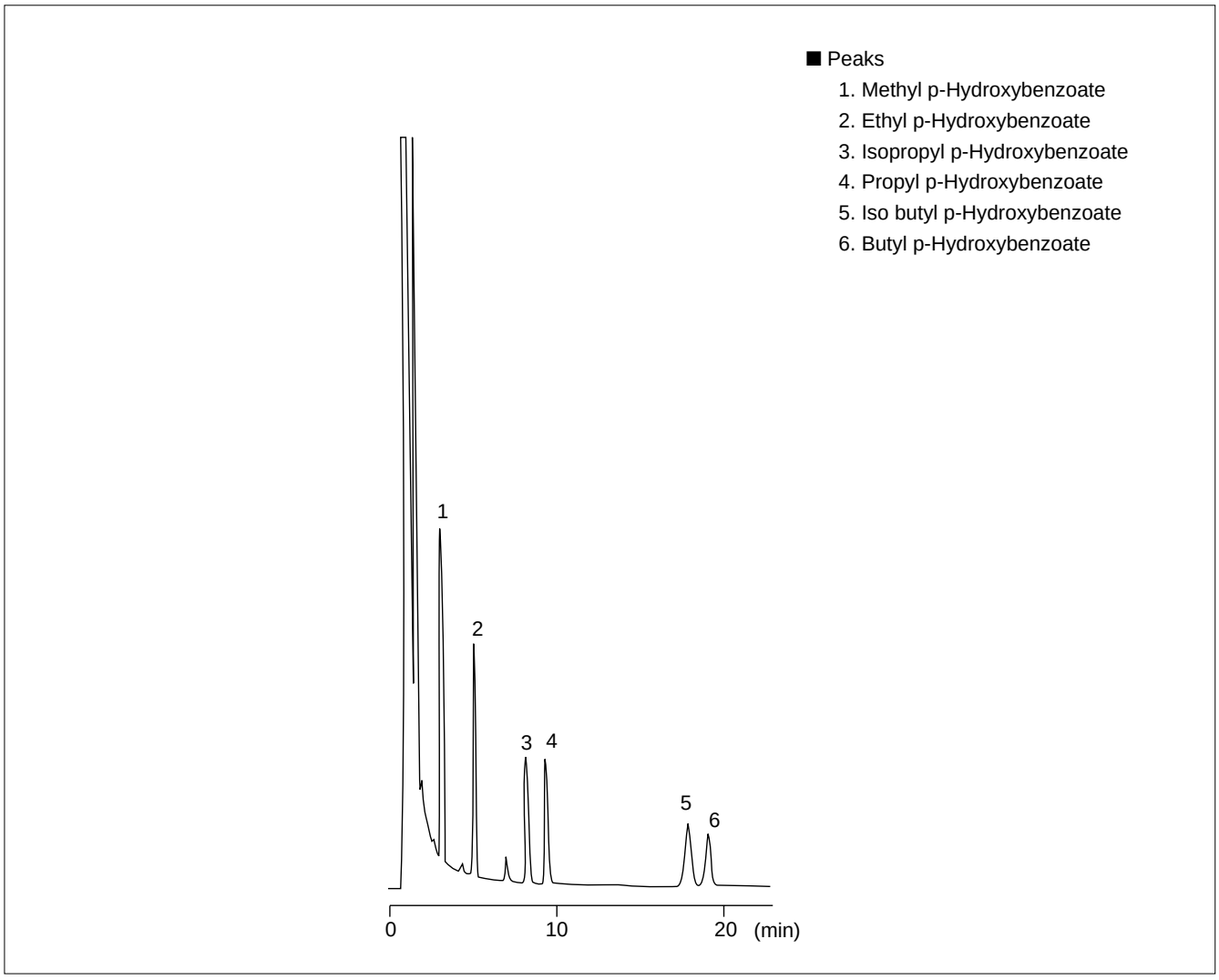


Fig. 3.1.1 Analysis of p-hydroxybenzoates in soy sauce

3.2 Analysis of Sorbic Acid in Ham - LC

■Pretreatment

1. A ham sample (1g) was homogenized with a 0.4N $HClO_4$ aqueous solution.
2. The solution was centrifuged. (12000r.p.m, 2min.)
3. 5 µL aliquot of the supernatant was injected.

■Analytical Conditions

Column	: Shim-pack CLC-ODS (150mmL. × 6.0mmI.D.)
Mobile Phase	: 10mM (Sodium) Phosphate Buffer (pH 6.9) / Acetonitrile = 20/1 (v/v)
Temperature	: 45°C
Flow rate	: 1.0mL/min
Detection	: UV 265nm

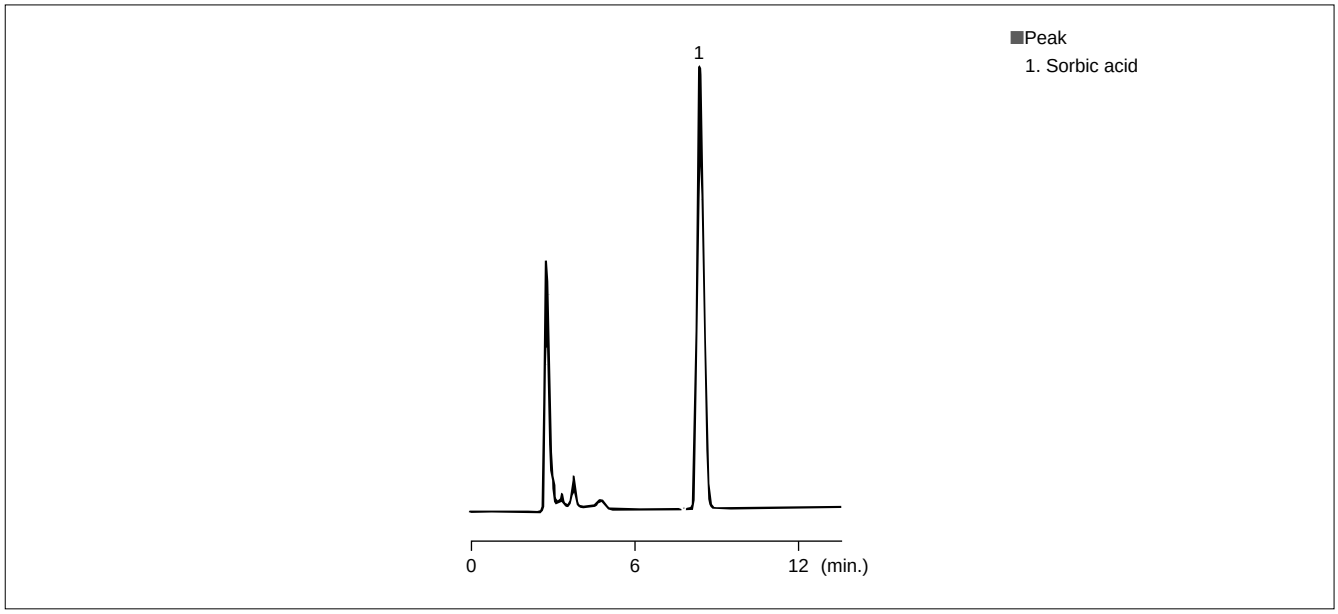


Fig. 3.2.1 Analysis of ham

3.3 Propionic Acid in Cookies and Bread - GC

■Explanation

Propionic acid is one of the components that form flavor and fragrance, included in fermented products such as miso, soy sauce and cheese as a microbial metabolite. It is also used as a preservative in cookies and bread because of its low toxicity and minimal effect on bread yeast.

When propionic acid is analyzed using GC with FID, the total calculation of the natural propionic acid, which is inherently included in the food, and the added propionic acid is obtained as the quantitative value.

References

1) Standard Methods of Analysis for Hygienic Chemists (annotation) 455 (1990), edited by the Pharmaceutical Society of Japan
2) Ministry of Health and Welfare (currently Ministry of Health, Labour and Welfare), Environmental Health Bureau, Food Sanitation Testing Policy, 33-35 (1989)

■Pretreatment

Propionic acid was extracted using steam distillation method.

■Analytical Conditions

Column	: 10%PEG6000 on shimalite TPA 1m × 3mm I.D.(glass)
Col. Temp.	: 150°C
Inj. Temp.	: 230°C
Det. Temp.	: 200°C(FID)
Carrier Gas	: N_2

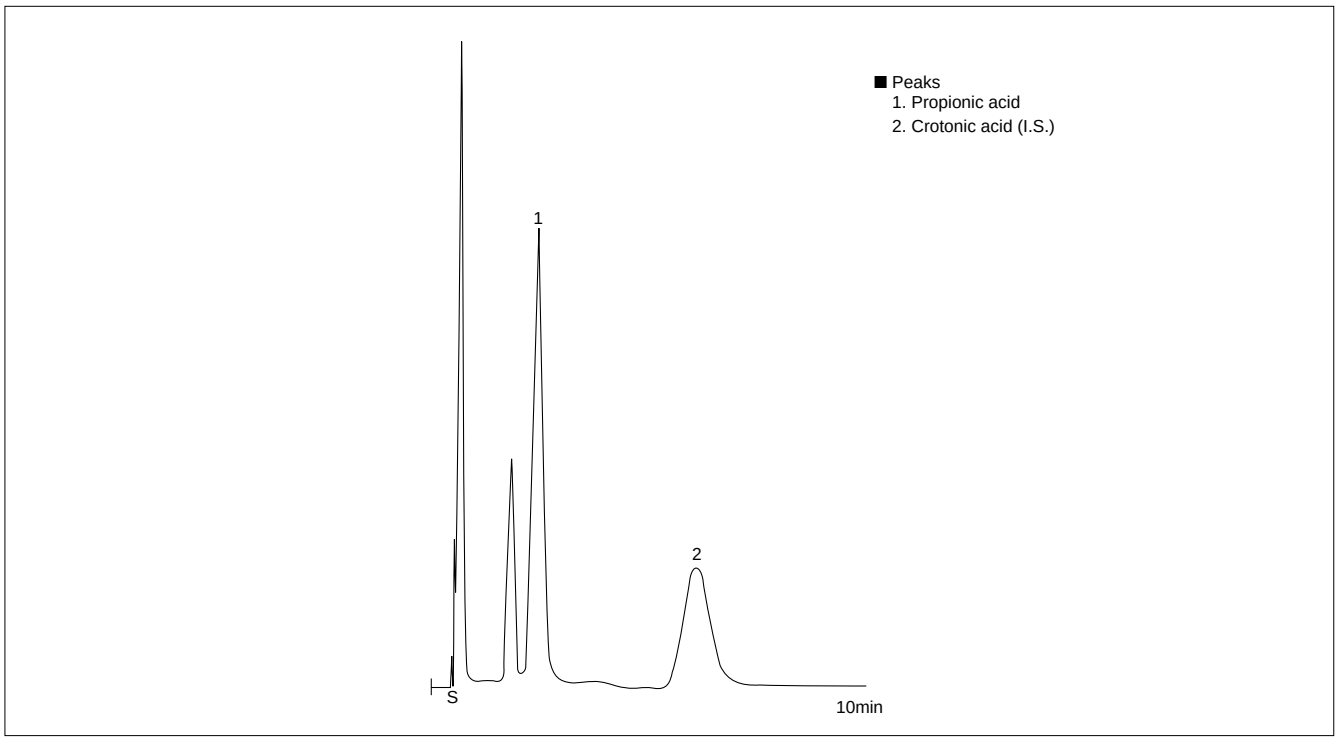


Fig. 3.3.1 Analysis of propionic acid

3.4 Sorbic Acid, Dehydroacetic Acid and Benzoic Acid - GC

■Explanation

The preservatives sorbic acid, dehydroacetic acid and benzoic acid are analyzed by UV absorption spectrum method or GC method. The UV method is fast and efficient but can be affected by coexisting substances such as fragrances, whereas GC has the advantage of being able to easily separate out such substances.

Here, these preservatives were extracted from a food product by direct extraction or steam distillation and refined to be analyzed by GC with FID.

References

Standard Methods of Analysis for Hygienic Chemists (annotation) 445 to 451 (1990), edited by the Pharmaceutical Society of Japan

■Pretreatment

1. Direct extraction

   Add saturated saline solution and sulfuric acid, homogenize with strong acidity and extract with ethyl ether. Reversely extract the ether layer using sodium hydrogen carbonate solution, re-extract using ethyl ether, and concentrate. Dissolve the residue with acetone, and GC aualysis is performed.

2. Steam distillation

   Pulverize the sample, add water, and neutralize pH. Add tartaric acid solution and sodium chloride and perform steam distillation. Extract residue using ethyl ether as previously described.

■Analytical Conditions

Column	: 5% DEGS+1%H_3PO_4 on chromosorb W 2m × 3mm I.D.(glass)
Col. Temp.	: 185°C
Inj. Temp.	: 230°C
Det. Temp.	: 250°C (FID)
Carrier Gas	: N_2

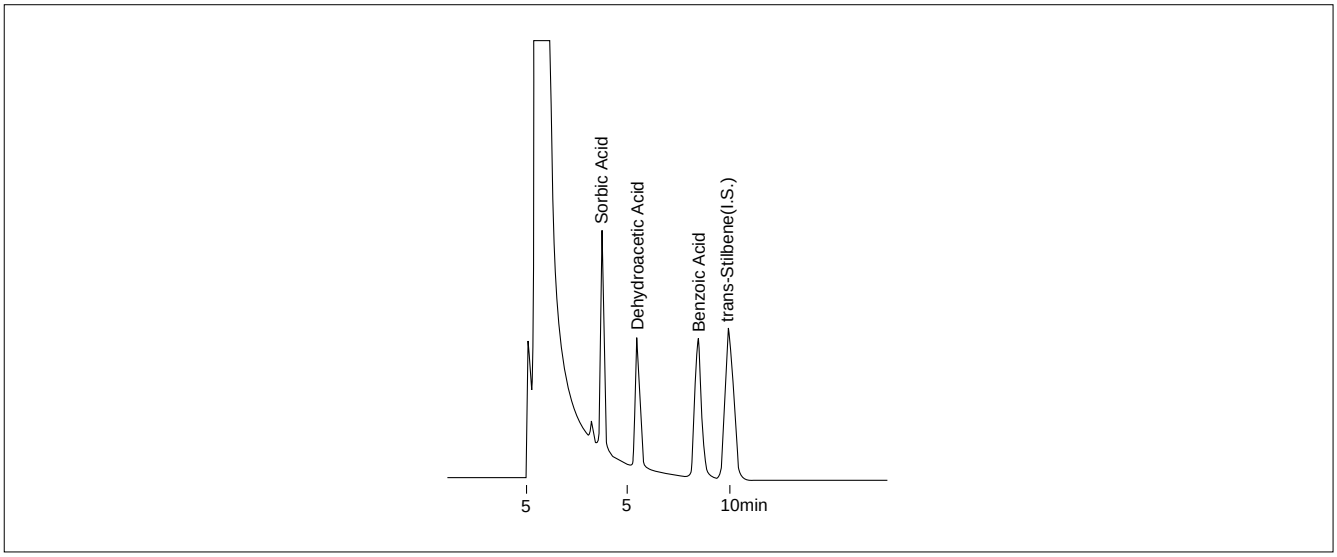


Fig. 3.4.1 Analysis of Preservatives

3.5 Analysis of Preservatives in Food Products with Absorption Photometry (1) - UV

■Explanation

Various preservatives are added to preservative and processed foods to prevent putrefaction and to keep freshness. The use of these food additives is strictly governed by the Food Sanitation Law to ensure that concentrations do not exceed the permitted safe concentrations for human consumption.

Here, preservatives in food products regulated by the Food Sanitation Law were analyzed with a Shimadzu double-beam spectrophotometer after pretreatment in accordance with the law.

■Pretreatment

- Sodium nitrite in a food product
  The sodium nitrite preservative in meat was separated by distillation, and sulfamic acid was diazotized using nitrite acid under acidity of hydrochloric acid, and colored with naphthylethylenediamine for measurement.
- Benzoic acid in a food product
  The benzoic acid preservative was separated and extracted from soy sauce using steam distillation in readiness for UV absorption measurement.
- Sorbic acid in a food product
  The sorbic acid preservative was separated and extracted from boiled fish paste using steam distillation in readiness for UV absorption measurement.
- Dehydroacetic acid in a food product
  The dehydroacetic acid preservative was separated and extracted from bean jam using steam distillation in readiness for UV absorption measurement.

■Analytical Conditions

Instrument	: UV Spectrophotometer
Reference	: blank
Solvent	: H_2O
Cell	: 10mm
Range	: 0 ~ 2Abs

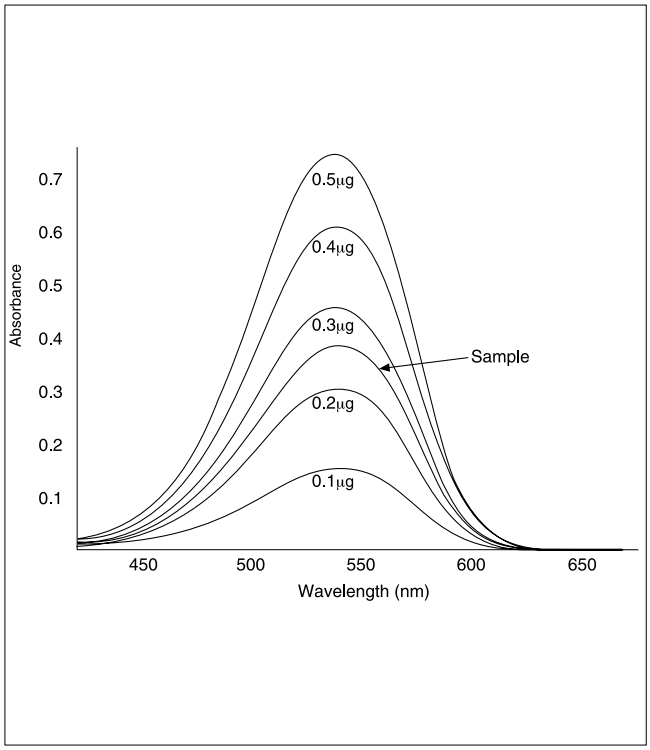


Fig. 3.5.1 Absorption spectrum for sodium nitrite

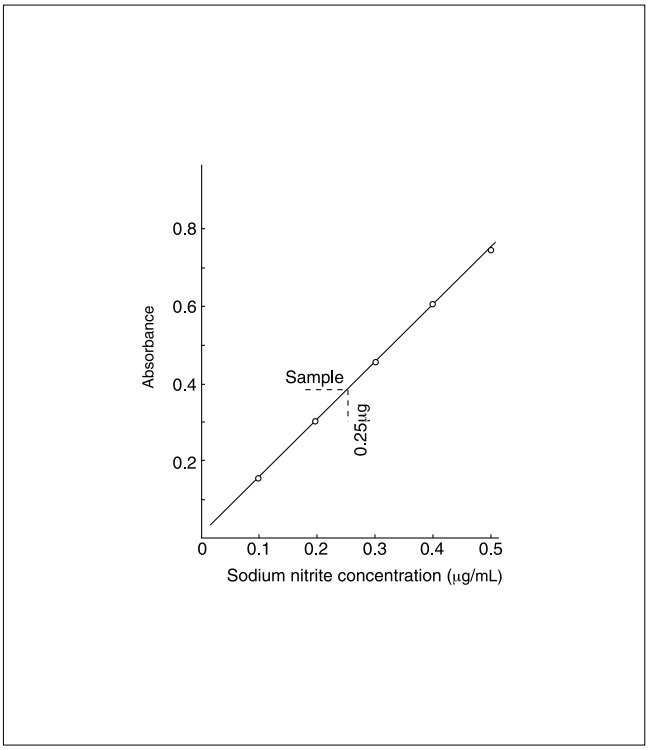


Fig. 3.5.2 Calibration curve for sodium nitrite

3.5 Analysis of Preservatives in Food Products with Absorption Photometry (2) - UV

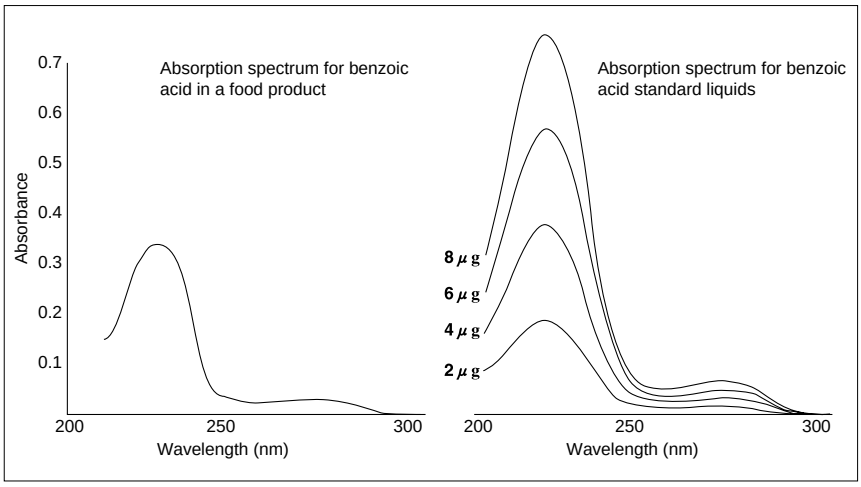


Fig. 3.5.3 Absorption spectrum for benzoic acid

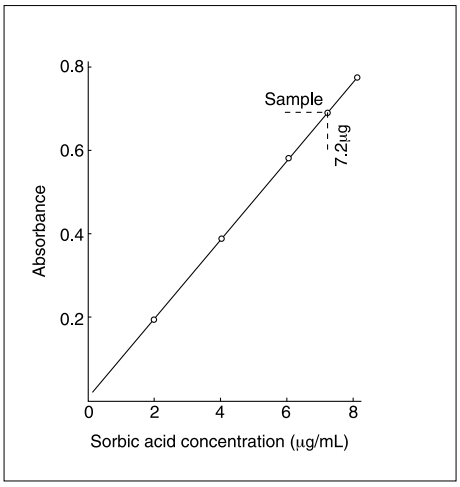


Fig. 3.5.4 Calibration curve for sorbic acid

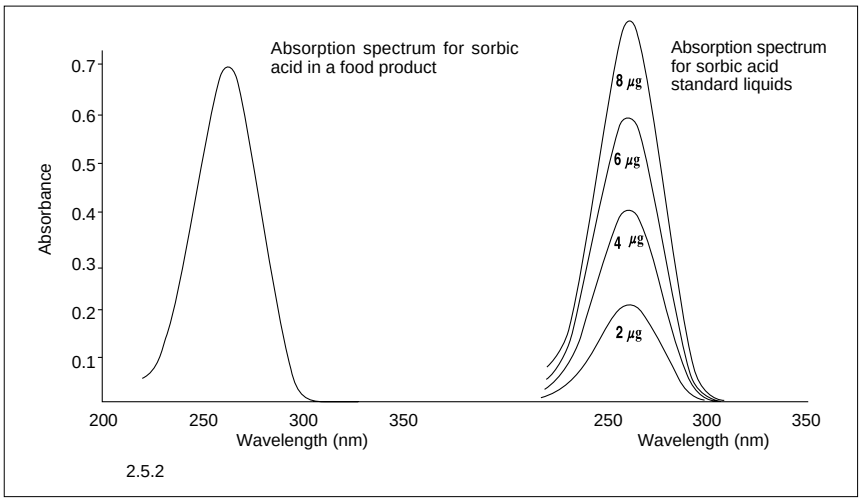


Fig. 3.5.5 Absorption spectrum for sorbic acid

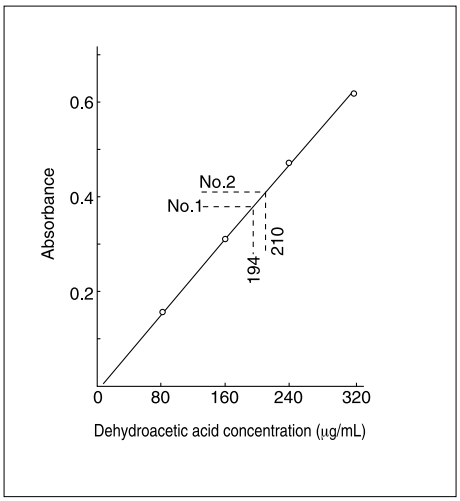
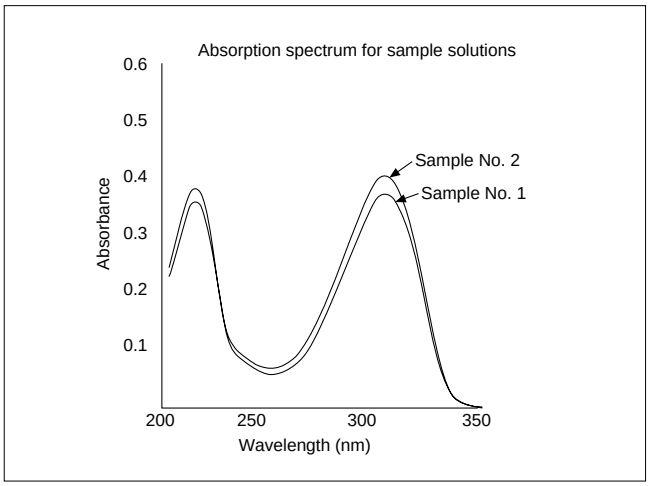


Fig. 3.5.6 Calibration curve for dehydroacetic acid



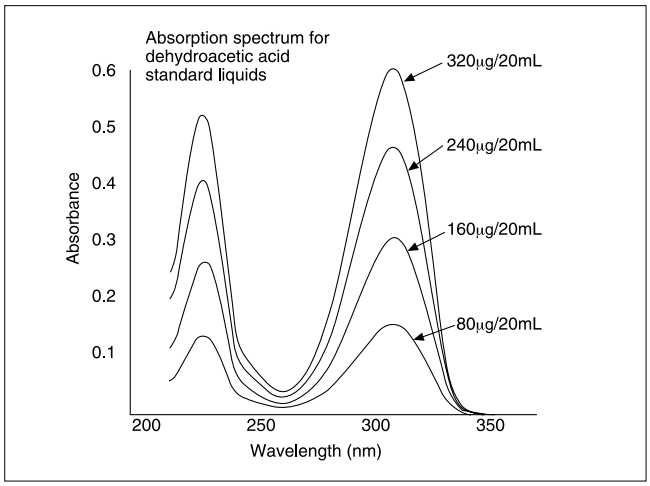


Fig. 3.5.7 Absorption spectrum for dehydroacetic acid

3.6 Analysis of Imazalil Contained in Oranges - LC

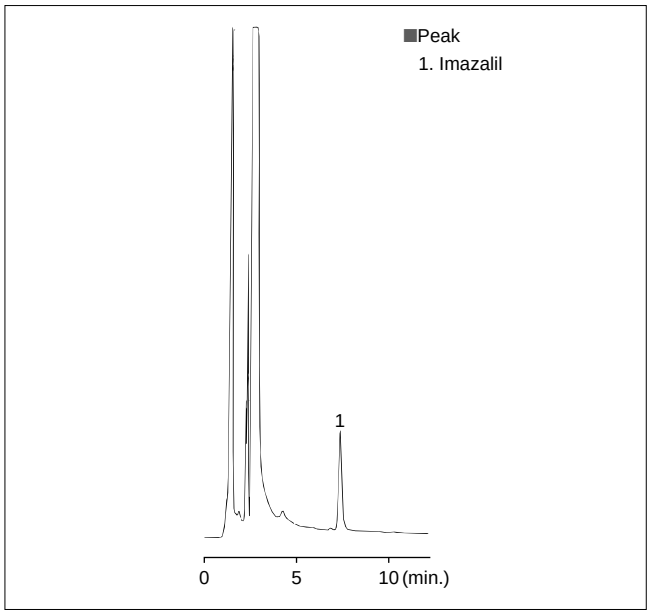


Fig. 3.6.1 Analysis of Imazalil in Oranges

■Analytical Conditions

Column	: STR ODS-II (150mmL. × 4.6mmI.D.)
Mobile phase	: 10 mM Sodium Phosphate Buffer (pH 6.9) / Acetonitrile = 45/55 (v/v)
Temperature	: 40°C
Flow rate	: 1.0mL/min
Detection	: UV 230nm

■Pretreatment

1. Extract orange peel with methylacetate under alkaline conditions.
2. Filter the extract through a membrane filter.
3. Inject 10 µL of the filtrate.

3.7 Analysis of Thiabendazole - GC

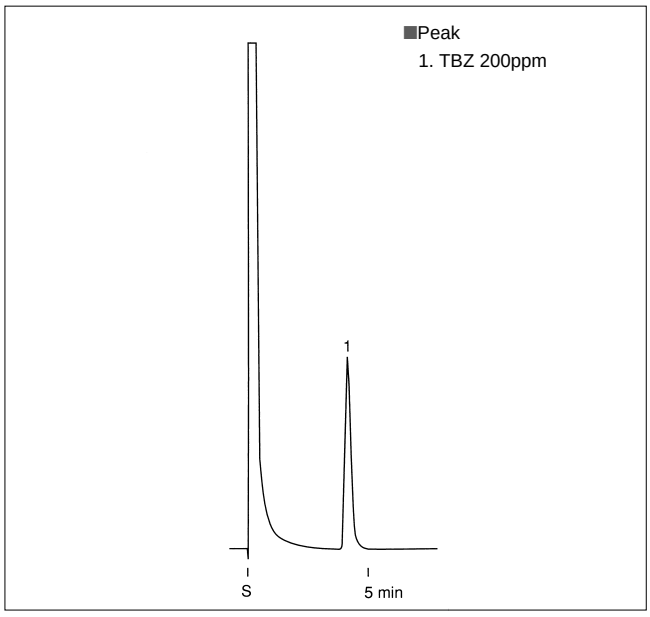


Fig. 3.7.1 Analysis of Thiabendazole

■Analytical Conditions

Column	: Thermon-3000 2% Chromosorb W (Shinwa Chemical Industries) 1.1m × 3.2mmI.D.
Carrier Gas	: N_2 60mL/min
Column temperature	: 220°C
Injection inlet temperature	: 270°C
Detection	: FID, 270°C

3.8 Analysis of EDTA in Mayonnaise - LC

■Explanation

EDTA in mayonnaise was analyzed after chelation of Fe ion. Reversed-phase ion pair chromatography with tetrabutylammonium ions was used for separation. In this analysis, a polymer column (ODP), instead of a silica column (ODS), was used because of the high pH of the mobile phase and the basicity of the tetrabutylammonium.
The following chromatogram shows the measurement of marketed mayonnaise with PDTA (the internal standard substance) and EDTA added.

References

Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)
Shimadzu Application News No. L214 (C190-E050)

■Pretreatment

1. Add chloroform to sample, mix together, and centrifugally separate (12000 r.p.m for 2 min, twice).
2. Add 0.01M $FeCl_3$ solution to water layer and mix together.
3. Inject 20µL of sample.

■Analytical Conditions

Column : Asahipak ODP-50 (150mmL. × 6.0mm I.D.)
Mobile phase : 20mM Sodium Phosphate Buffer (pH 6.9) containing 10mM Tetrabutylammonium Hydrogensulfate (adjust to pH 7.5 with 4M of NaOH)
Temperature : 40°C
Flow rate : 0.8mL/min
Detection : UV 255nm

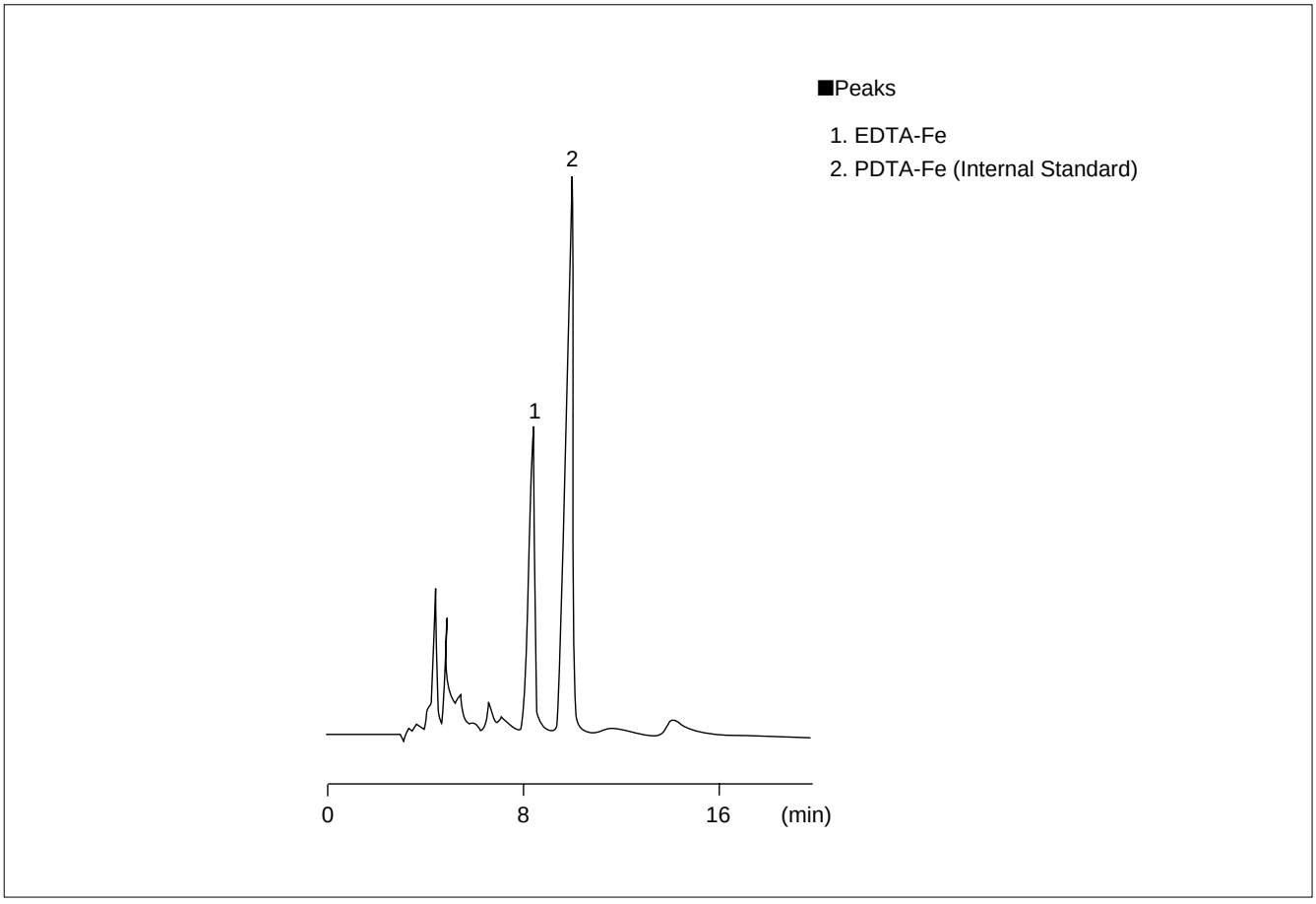


Fig. 3.8.1 Analysis of EDTA in mayonnaise

3.9 Analysis of Cooking Oils - LC

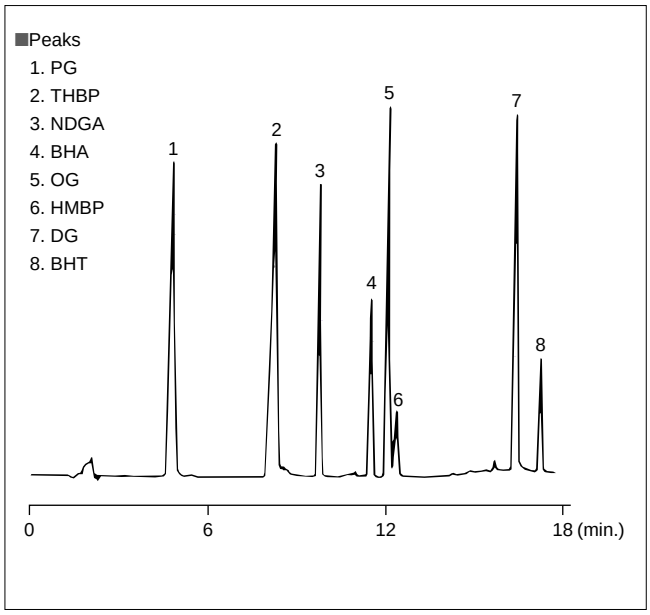


Fig. 3.9.1 Analysis of Cooking Oil
(with standard phenol antioxidant added)

■Analytical Conditions

Column : STR ODS-II (150mmL. × 4.6mmI.D.)
Mobile phase : Gradient elution
A: 10mM Sodium Phosphate Buffer (pH 2.6)
B: Acetonitrile
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : UV 230nm

■Analytical Conditions

Time	B Conc.
0.00 min (Initial conditions)	30%
4.00 min	30%
4.01 min	63%
10.00 min	63%
10.01 min	90%
16.00 min	90%
16.01 min	30%
25.00 min	30%

■Pretreatment

1. Add 1mL methanol to 100 µL cooking oil (with 100 µg/mL standard sample added) and agitate the mixture.
2. After centrifugal separation, inject 20 µL of the upper layer.

3.10 Analysis of Antioxidant - GC

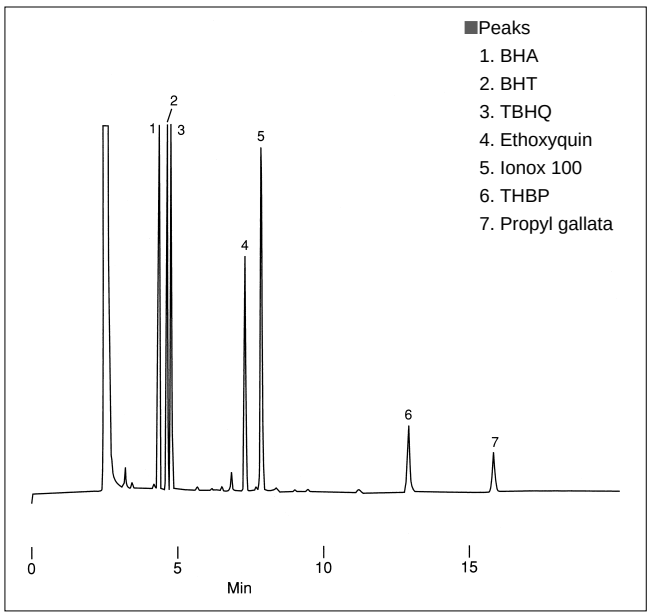


Fig. 3.10.1 Analysis of Antioxidant
(Reprinted from 1997 SUPELCO Chromatography Product General Catalogue Japanese Version)

■Analytical Conditions

Column : SAC-5 (Supelco)
30m × 0.25mmI.D. df = 0.25µm
Carrier Gas : He 30cm/sec
Column temperature : 200°C
Detection : FID, 250°C
Injection method : Split (1:100)
Injection volume : 2 µL (200 µg/mL for each)

3.11 Analysis of FDA-Regulated Antioxidants - GC

■Analytical Conditions

Column : Rtx-50 (Restek)
30m × 0.53mmI.D. df = 0.5µm
Carrier Gas : He 89cm/sec
Column temperature : 165°C (1min) -10°C/min -310°C
Injection method : OCI
Detection : FID
Injection volume : 1 µL (100 ppm for each)

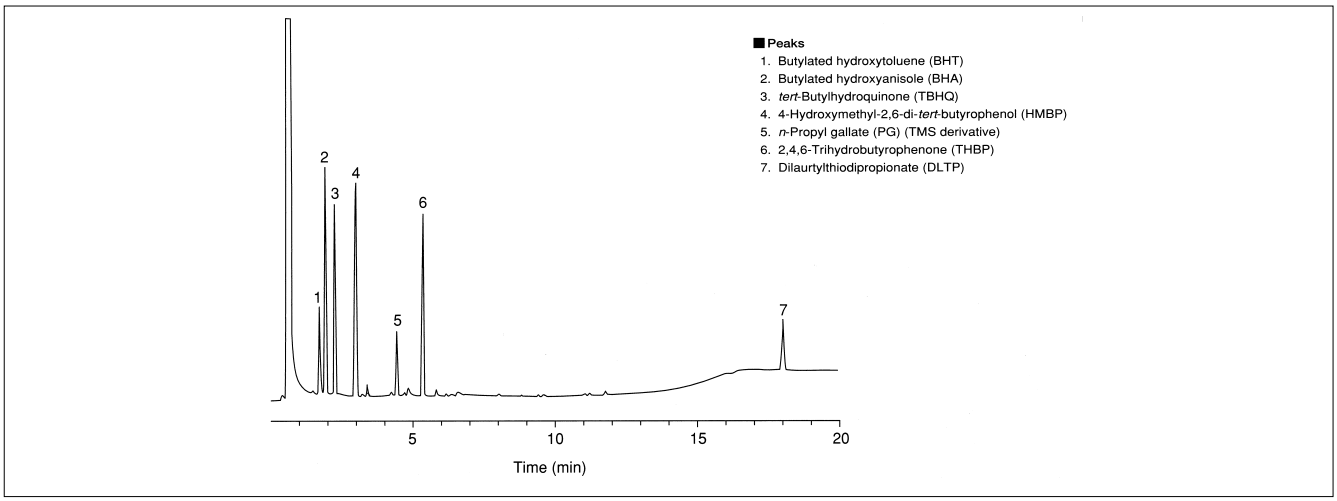


Fig. 3.11.1 Analysis of FDA-Regulated Antioxidants
(Reprinted from J. of Chromatographic Science, Vol. 33, July 1995)

3.12 Analysis of Tocopherol - GC

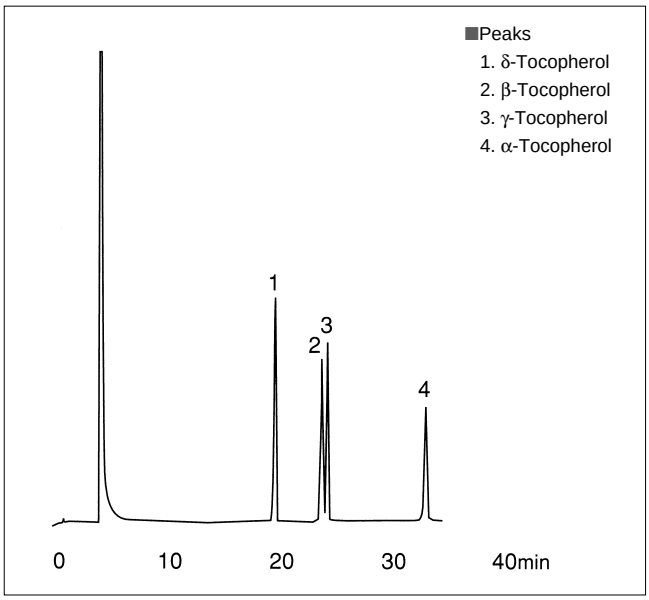


Fig. 3.12.1 Analysis of Tocopherol

■Analytical Conditions

Column :CBP5-M25-050 (Shimadzu)
25m × 0.22mmI.D. df = 0.25µm
Carrier Gas : He 0.6mL/min
Column temperature : 250°C
Injection method : Split 300°C
Detection : FID 300°C

3.13 Analysis of Phenol Antioxidant in Foods - LC

■ Explanation

The exposure of food constituents to oxygen in the air leads to the creation of oxidation products and deterioration in quality. To prevent this, various antioxidants are used as food additives. Here, we will be looking at how phenol antioxidants, which are used particularly often in oil products, can be analyzed with HPLC.

There are four types of phenol antioxidant that are approved as food additives in Japan: BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), NDGA (nordihydroguaiaretic acid), and PG (propyl gallate). They are authorized for use in oil, fat, and butter, as well as frozen and dried seafood products.

The analysis of nine phenol antioxidants, the four mentioned above and five that are used in other countries, is described here as an example.

■ Analysis of Butter

The results of analyzing butter after performing the pretreatment described on the right are shown in Fig. 3.13.1. The lower line represents the result of analyzing butter and the upper line represents the result of analyzing butter after adding 20mg/L of the nine phenol antioxidants at the pretreatment stage.

■ Analytical Conditions

Column : Shim-pack FC-ODS (75mmL. × 4.6mm I.D.)
Mobile phase : A: 5% Acetic Acid Solution
B: Methanol/Acetonitrile = 1/1 (v/v)
B 40% → 80% /15min
Linear Gradient
Flow rate : 1.0mL/min
Temperature : 40˚C
Detection : SPD-10A*VP* 280nm

■ Pretreatment

0.5g of butter
↓ ← 1g of sodium sulfate
5mL of acetonitrile/isopropanol/ethanol = 2/1/1 (v/v/v)
Mixing
↓
Left for 1 hour at -20˚C
↓
Centrifugation at 3,000rpm for 5min
↓
Supernatant
↓
Filtration
↓
10μL injected

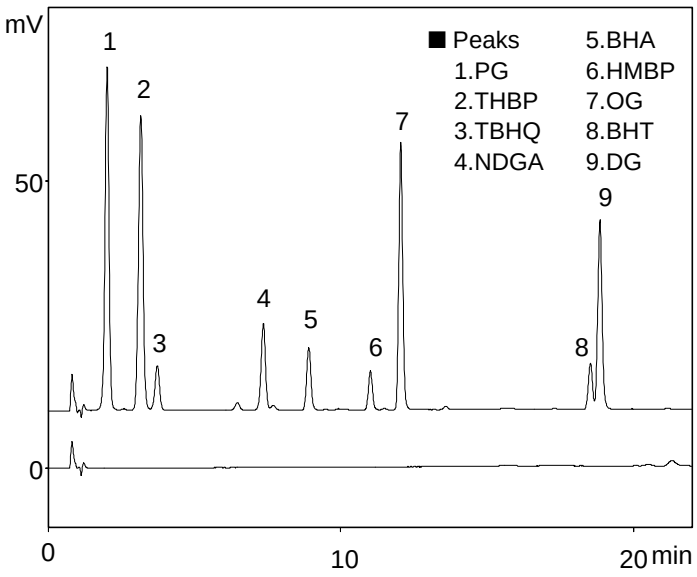


Fig. 3.13.1 Analysis of butter

3.14 Analysis of Sweetener in Soft Drink - LC

■Explanation

This is an example of simultaneous analysis of the sweeteners aspartame, saccharine, benzoic acid, sorbic acid and glycyrrhizic acid.

References

Shimadzu HPLC Food Analysis Applications Data Book (C190-E047)

■Analytical Conditions

Column	: STR ODS-M(150mmL. × 4.6mmI.D.)
Mobile phase	: 40mM Sodium Acetate Buffer (pH 4.0)/ Methanol = 3/1 (v/v)
Temperature	: 40°C
Flow rate	: 1.0mL/min
Detection	: UV 250nm

■Pretreatment

A soft drink was directly injected without pretreatment.

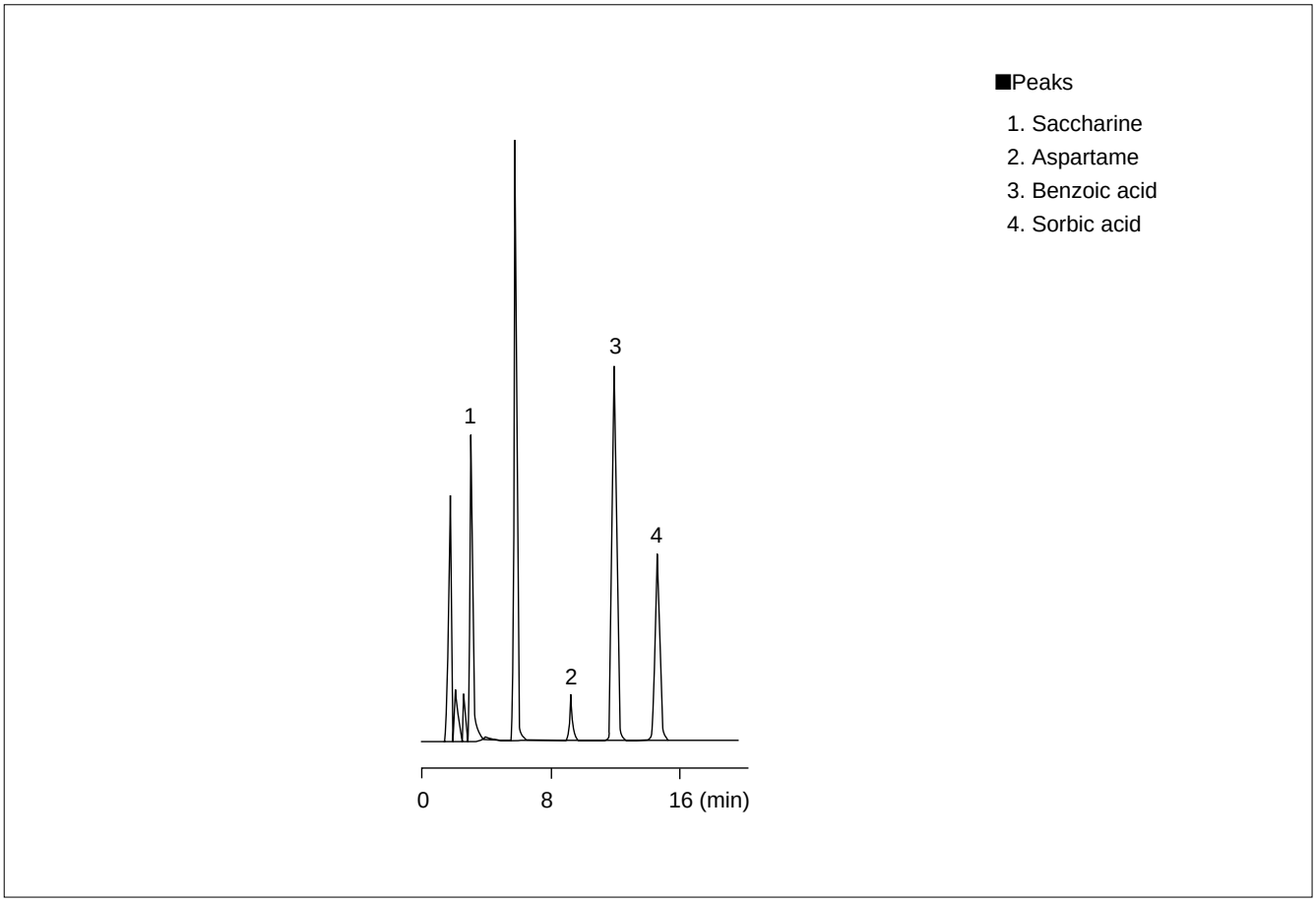


Fig. 3.14.1 Analysis of sweetener in soft drink

3.15 Analysis of Saccharin in Pickles - LC

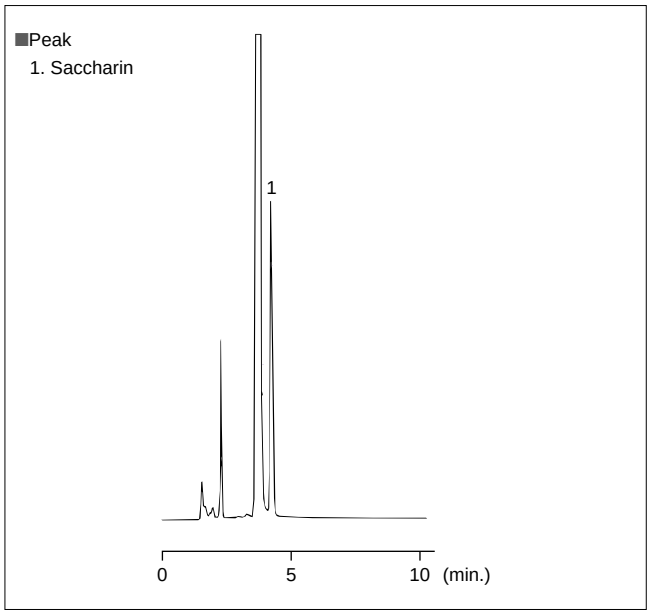


Fig. 3.15.1 Analysis of Saccharin in Pickles

■Analytical Conditions

Column	: STR ODS-II (150mmL. × 4.6mmI.D.)
Mobile phase	: 10 mM Sodium Phosphate Buffer (pH 6.9) / Acetonitrile = 80/20 (v/v)
Temperature	: 40°C
Flow rate	: 1.0mL/min
Detection	: UV 254nm

■Pretreatment

1. Grind 3 g of pickles.
2. Add 50 mL sodium phosphate buffer (pH 6.9) and heat for 10 minutes at 40°C.
3. Dilute supernatant by five times with pure water and filter it through a membrane filter.
4. Inject 10 µL of the filtrate.

3.16 Analysis of Acesulfame K - LC

This is an example of analyzing a standard sample of acesulfame K. Though not approved for use in Japan yet, it is widely used in Europe and the United States.

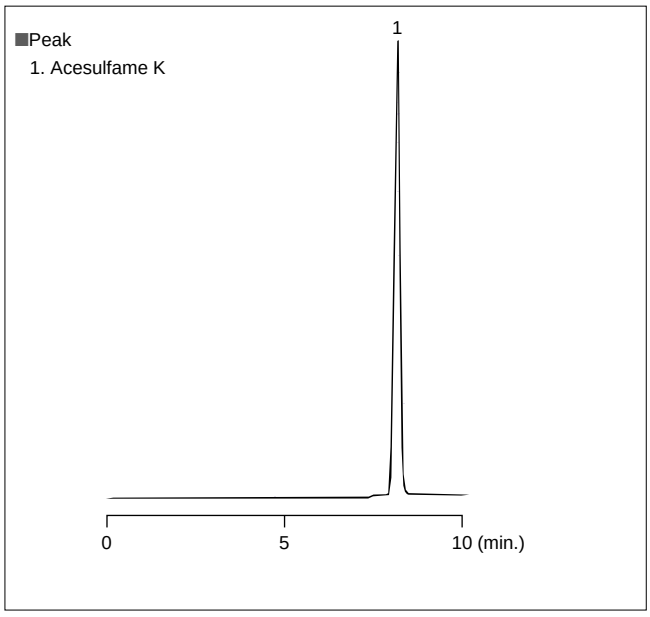


Fig. 3.16.1 Analysis of an Acesulfame K Standard

■Analytical Conditions

Column	: STR ODS-II (150mmL. × 4.6mmI.D.)
Mobile phase	: 20 mM Sodium Phosphate Buffer (pH 6.9)
Temperature	: 45°C
Flow rate	: 1.5mL/min
Detection	: UV 227nm

3.17 Saccharine and Sodium Saccharine - GC

■Explanation

Saccharine and sodium saccharine are used as artificial sweeteners. Saccharine is only used in chewing gum because it does not dissolve easily in water whereas sodium saccharine does and is widely used in pickles and jams.
Saccharine and sodium saccharine are extracted from food products and refined, and after being methylated, they are analyzed by GC with FID or FPD. Here, a GC with FID analysis example will be introduced.

References

Standard Methods of Analysis for Hygienic Chemists (annotation) 493 to 495 (1990), edited by the Pharmaceutical Society of Japan.

■Pretreatment

1. Extract and refine sample by dialysis extraction or direct extraction.
2. Produce a derivative (methylate) of saccharine using diazomethane, etc.
3. Dissolve in ethyl acetate, etc. and use this liquid as the sample.

■Analytical Conditions

Column	: SE-30 5% on chromosorb W 1.5m × 3mm I.D. (glass)
Col. Temp.	: 190°C
Inj. Temp.	: 250°C
Det. Temp.	: 230°C (FID)
Carrier Gas	: N_2

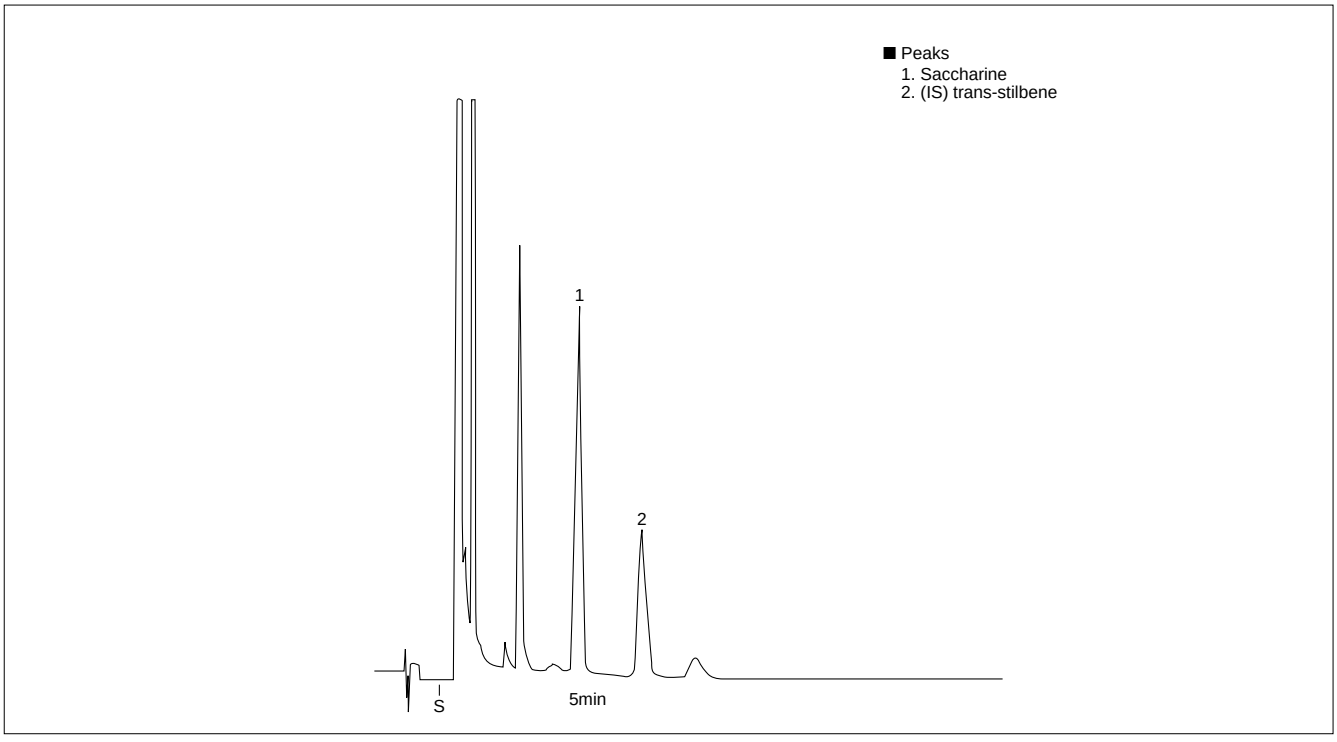


Fig. 3.17.1 Analysis of saccharine

3.18 Simultaneous Analysis of Water-soluble Tar Pigments - LC

■Explanation

Synthetic and natural compounds are used as food pigments, and HPLC is a powerful tool for analyzing such compounds. The photodiode array analysis, which allows simultaneous analysis at multiple wavelengths and spectrum display, further facilitates the analysis and identification of unknown components.

Here, a simultaneous analysis example for water-soluble tar pigments will be introduced showing multi chromatograms for each absorption wavelength using a photodiode array detector.

References

Masaaki Ishikawa et al; Summary of the 31st Annual Conference of the Japan Hygienic Chemistry Council (1994)

■Analytical Conditions

IColumn : STR ODS-II (150mmL. × 4.6mmI.D.)
Mobile phase : A: 20mM Ammonium Phosphate Buffer (pH 6.8)/Isopropanol = 25/1 (v/v)
B: Acetonitrile
Gradient Elution
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : Photodiode Array detection 220nm to 700nm

■Gradient Conditions

Time	B concentration
0.00 min (initial condition)	0%
15:00 min	20%
45.00 min	40%
55.00 min	70%
55.01 min	0%
65.00 min	0%

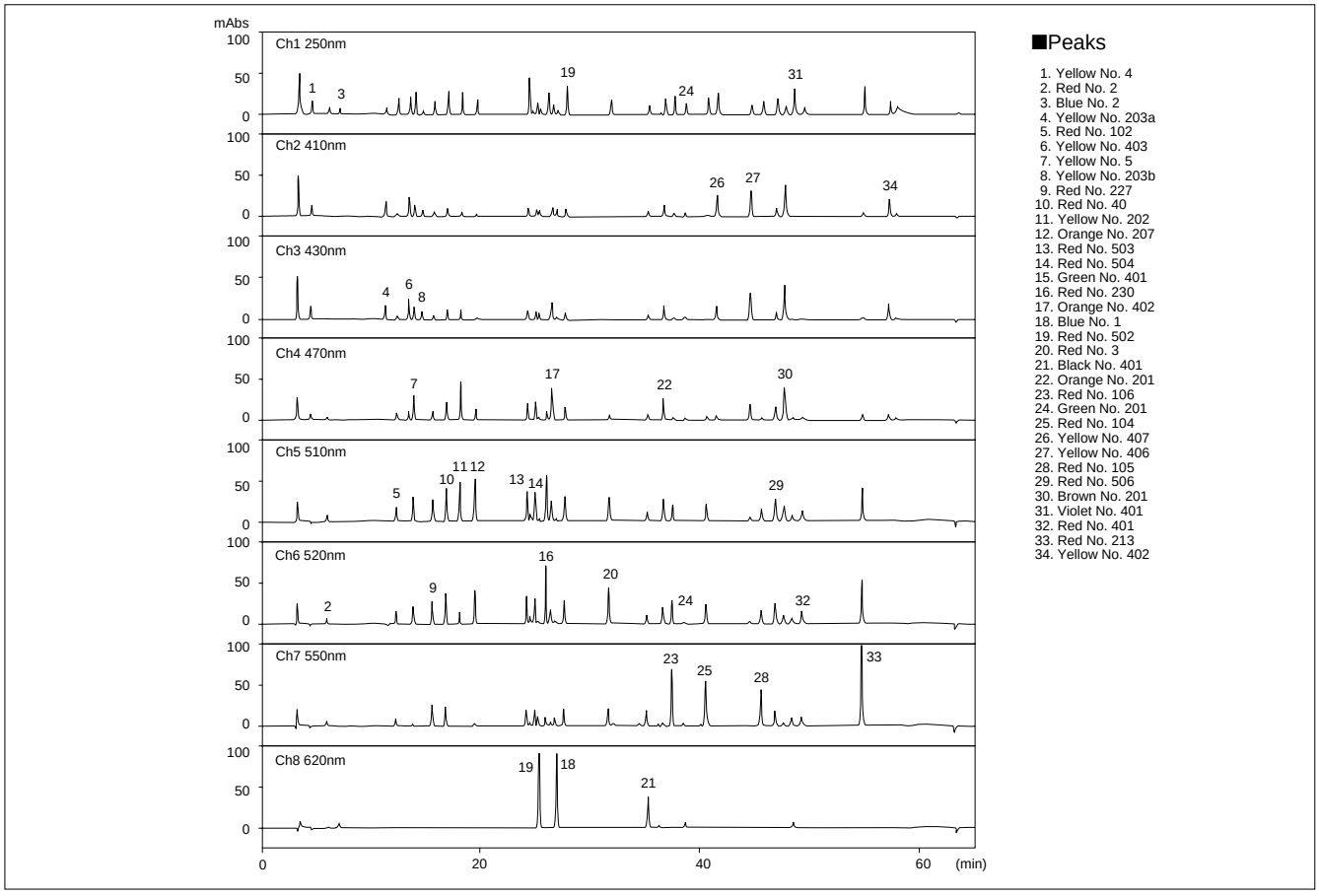


Fig. 3.18.1 Simultaneous analysis of water-soluble tar pigments

3.19 Analysis of synthetic coloring agents in Japanese Fukushinzuke pickles, pickled radish and candy - LC

The following figures show chromatograms of synthetic coloring agents in Japanese Fukushinzuke pickles, pickled radish and candy.

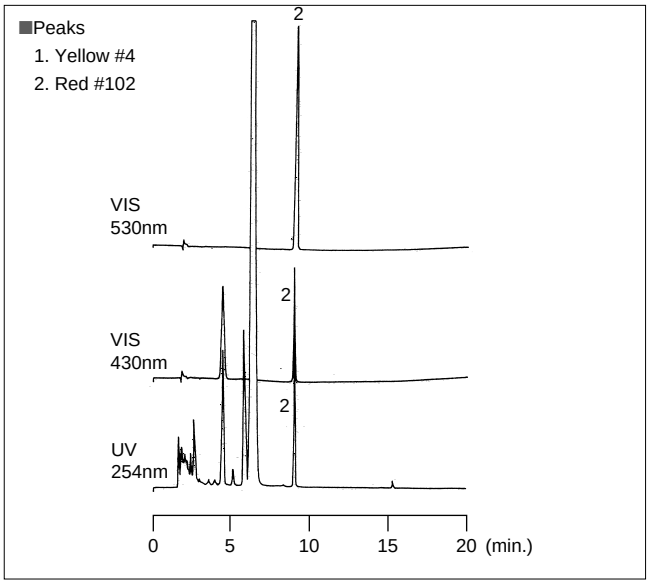


Fig. 3.19.1 Analysis of Japanese *Fukushinzuke* Pickles

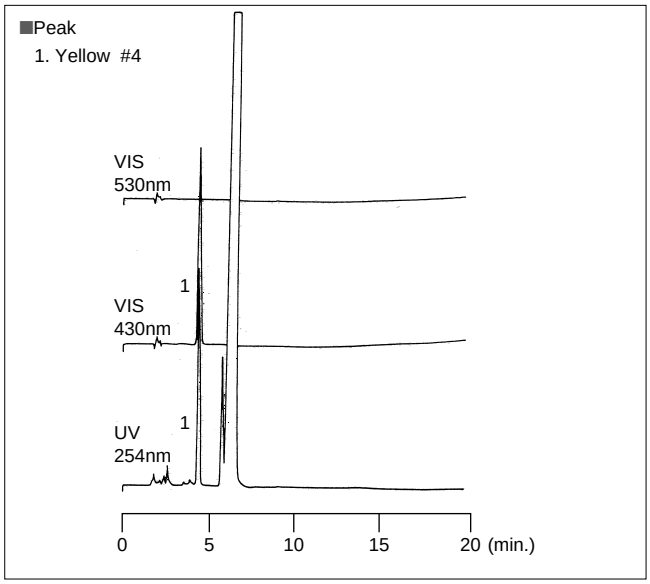


Fig. 3.19.2 Analysis of Pickled Radish

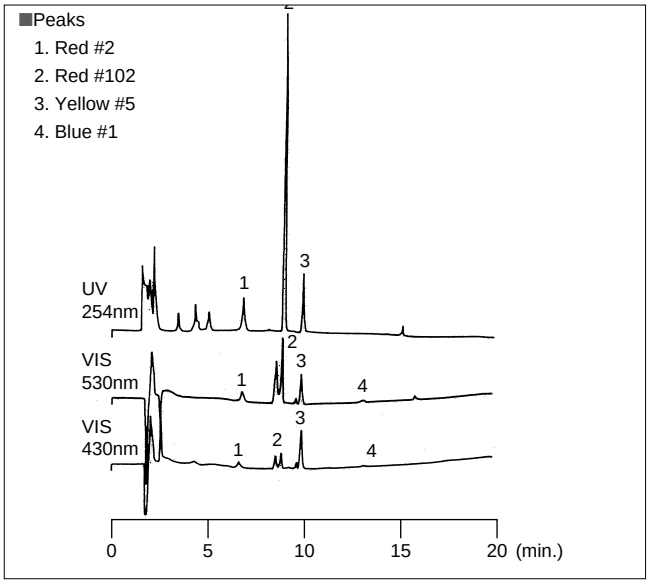


Fig. 3.19.3 Analysis of Candy

■Analytical Conditions

Column : Shim-pack CLC-ODS (150mmL. × 6.0mm I.D.)
Mobile Phase : Gradient elution
A: 10mM (Ammonium) Phosphate Buffer (pH 7.0)
B: Methanol
Temperature : 50°C
Flow Rate : 1.5mL/min

■Gradient Time Program

Time	B Conc
0.00 min (Initial)	0%
15.00 min	90%
19.00 min	100%
19.01 min	0%
30.00 min	0%

3.20 Analysis of Blue #1 in candy - LC

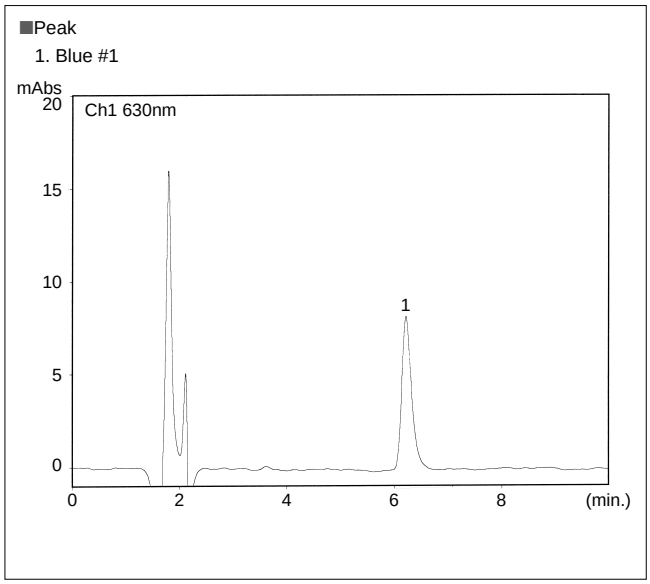


Fig. 3.20.1 Analysis of Blue #1 in Candy

■Analytical Conditions

Column : STR ODS-II (150mmL. × 4.6mm I.D.)
Mobile Phase : A: 10mM(Sodium) Phosphate Buffer (pH 2.6) containing 100mM Sodium Perchlorate
B: Acetonitrile
A / B = 10 / 3 (v/v)
Temperature : 40°C
Flow Rate : 1.0mL/min

■Pretreatment

1. A candy sample (5.5g) was extracted with water (20mL) by stirring.
2. After filtration, a 20µL aliquot of the filtrate was injected.

In Fig. 3.20.2, the spectrum of the Blue #1 standard is shown overlaid on the Blue #1 peak from Fig. 3.20.1.

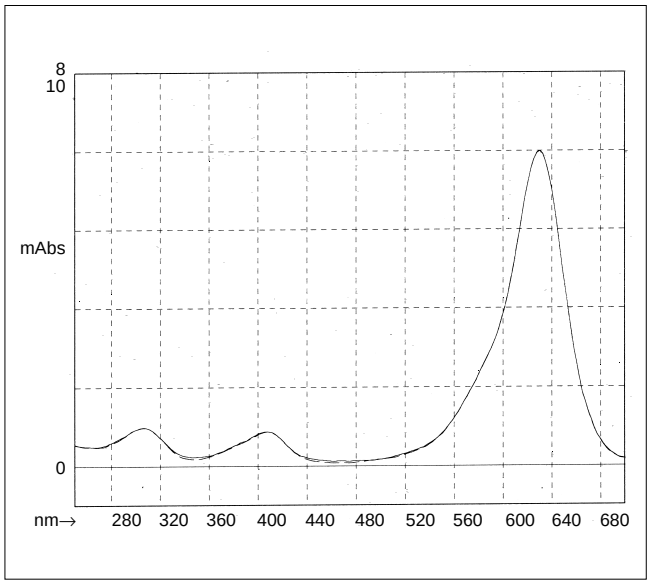


Fig. 3.20.2 Spectrum of Blue #1 (standard spectrum overlaid)

3.21 Analysis of curcumin - LC

The following page shows multiple chromatograms of curcumin (an extract of Curcuma Longa L.), a yellow coloring agent, and Shikonin (an extract of Lithospermum erthrohizon), a purple coloring agent.

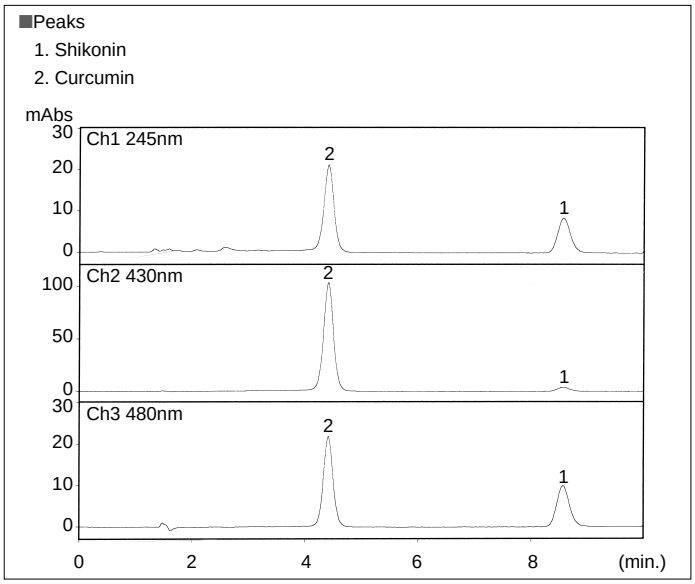


Fig. 3.21.1 Multi-chromatogram of Shikonin and Curcumin

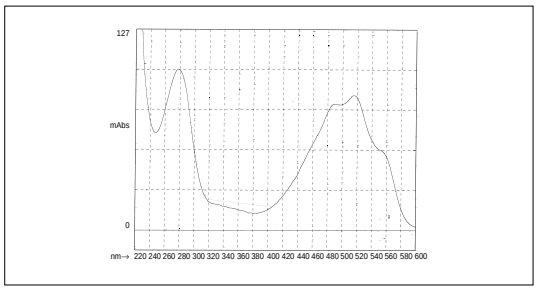


Fig. 3.21.2 Spectrum of Shikonin

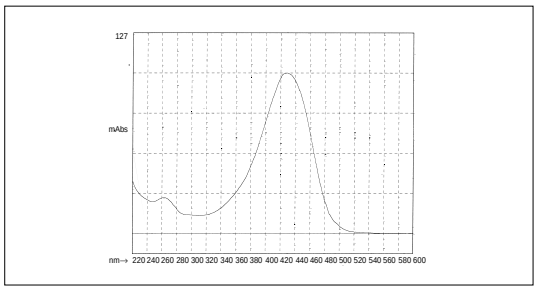


Fig. 3.21.3 Spectrum of Curcumin

Fig. 3.21.4 shows a chromatogram of curcumin in candy. A comparison of spectra reveals that any impurities with a UV absorption near the lower wavelength region are hidden behind the curcumin peak.

■Analytical Conditions

Column : STR ODS-II (150mmL. × 4.6mm I.D.)
Mobile Phase : 10mM (Sodium) Phosphate Buffer (pH 2.6) / Methanol = 3 / 7 (v/v)
Temperature : 40°C
Flow Rate : 1.2mL/min
Detection : Photodiode array (220nm - 700nm)

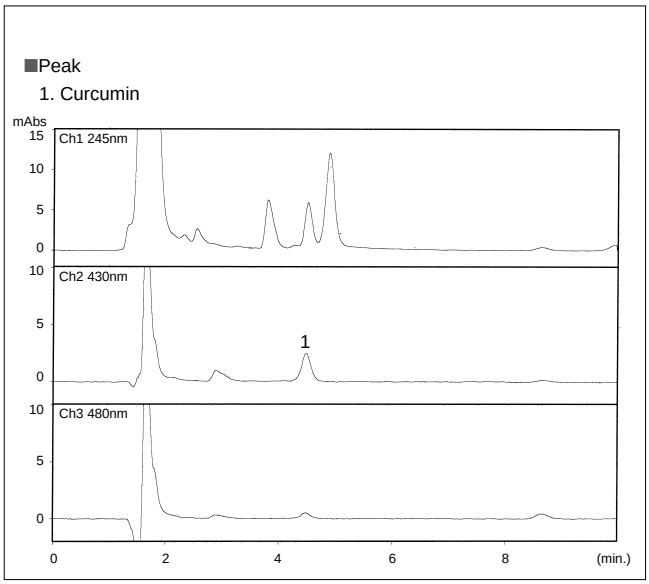


Fig. 3.21.4 Analysis of Candy

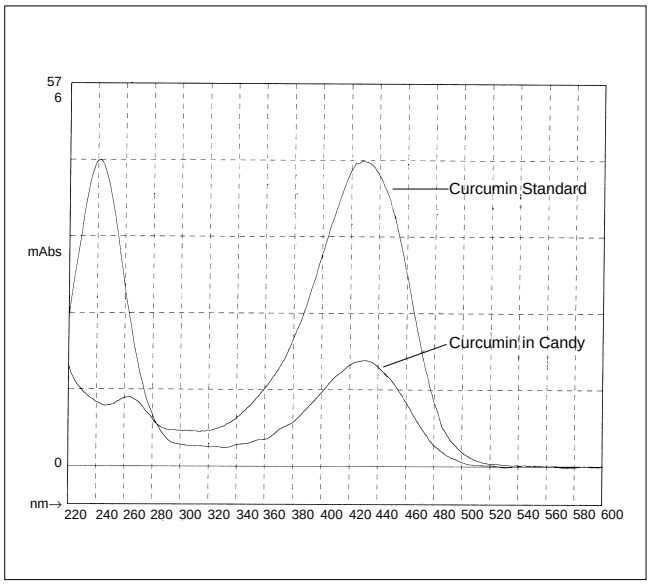


Fig.3.21.5 Spectra of Curcumin Standard and Curcumin in Candy

3.22 Analysis of Flavonoid Pigments - LC

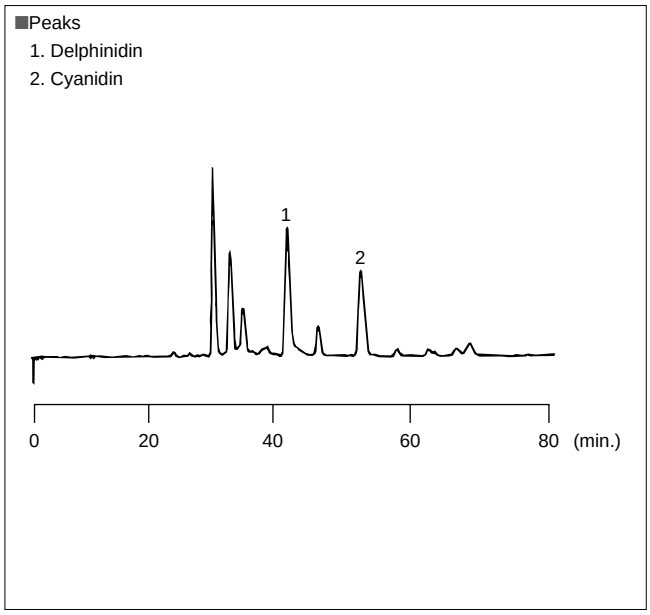


Fig. 3.22.1 Analysis of Flavonoid Pigments

■Analytical Conditions

Column : Zorbax ODS (250mmL. × 4.6mmI.D.)
Mobile phase : A: 0.5% Phosphoric Acid Aq. Solution
B: Methanol
A / B = 95 / 5 ~ 40 / 60 1% / min. Linear Gradient
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : VIS 530nm

3.23 Analysis of Betanin - LC

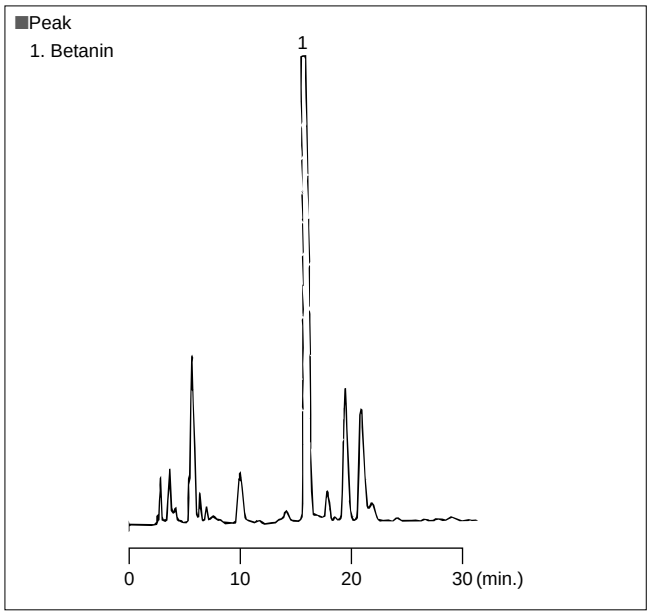


Fig. 3.23.1 Analysis of Betanin

■Analytical Conditions

Column : Zorbax C8 (250mmL. × 4.6mmI.D.)
Mobile phase : A / B = 100 / 0 ~ 60 / 40 1% / min. Linear Gradient
A: 10mM (Sodium) Phosphate Buffer (pH 2.6)
B: Methanol
Temperature : 40°C
Flow rate : 1.0mL/min
Detection : VIS 535nm

3.24 Analysis of Food Fragrances - GC

■Explanation

This section shows an example of analyzing fragrances added to candy or confections using the headspace method. Examples of analyzing confections are also shown.

■Pretreatment Method

Appropriate amounts of standard fragrance sample and confections were placed in vials and heated at 130°C for 40 minutes.

■Analytical Conditions

Column	: CBP1 25m × 0.53mmI.D. df = 3.0µm
Column temperature	: 90°C -6°C/min -230°C
Injection inlet temperature	: 300°C
Detector temperature	: 300°C (FID)
Carrier Gas	: He (4.3mL/min)
Injection method	: Direct Injection
Injection volume	: 0.8mL

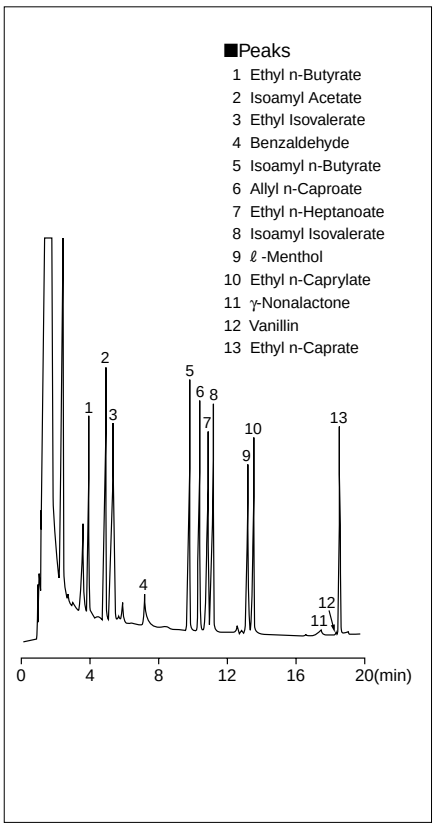


Fig. 3.24.1 Analysis of Standard Fragrance Additives

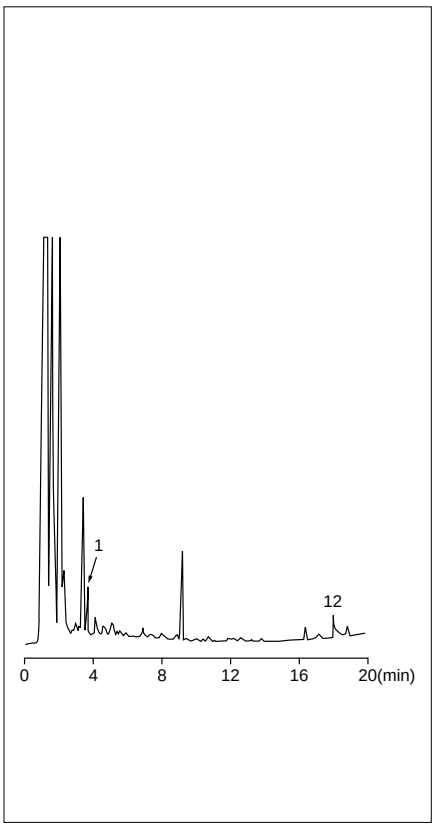


Fig. 3.24.2 Analysis of Cookie (6.4 g)

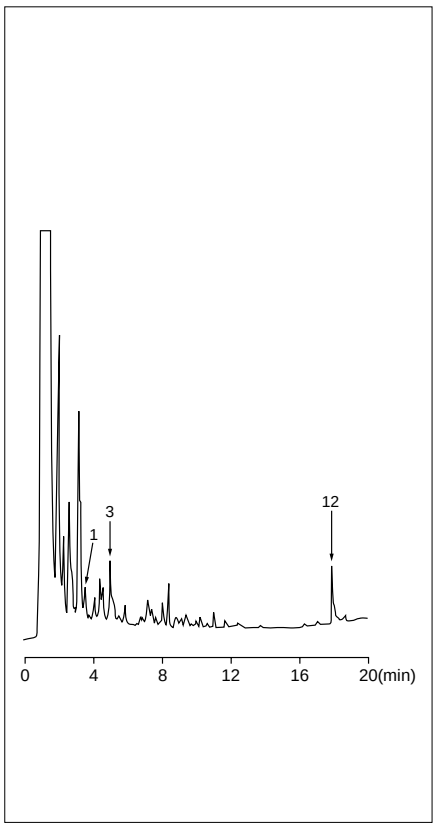


Fig. 3.24.3 Analysis of Chocolate (nut cream filled) (3.9 g)

3.25 Analysis of Standard Fragrance Sample (1) - GC

■Analytical Conditions

Column	: Rtx-1 (Restek) 60m × 0.53mmI.D. df = 0.5µm
Carrier Gas	: He 20cm/sec
Column temperature	: 70°C (15min) -2°C/min -190°C
Injection method	: Split (1:20), 220°C
Detection	: FID, 260°C

■Peaks

1. methanol
2. acetaldehyde
3. ethanol
4. acetone
5. isopropyl alcohol
6. methylene chloride
7. hexane
8. ethyl acetate
9. ethyl propionate
10. n-hexanal
11. ethylbutyrate
12. furfural
13. trans-2-hexenal
14. α-thujone
15. α-pinene
16. camphene
17. sabinene
18. β-pinene
19. octanal
20. myrcene
21. α-phellandrene
22. α-terpinene
23. *p*-cymene
24. δ-limonene
25. γ-terpinene
26. octanol
27. terpinolene
28. nonanal
29. linalool
30. cis-limonene monoxide
31. trans-limonene monoxide
32. citronellal
33. terpinene-4-ol
34. α-terpineol
35. decanal
36. D&L carveol
37. neral
38. carvone
39. geranial
40. neryl acetate
41. gerany acetate
42. α-ionone
43. β-caryophyllene
44. trans-α-bergamotene
45. BHA
46. β-ionone
47. valencene
48. γ-elemene
49. β-bisabolene
50. nootkatone

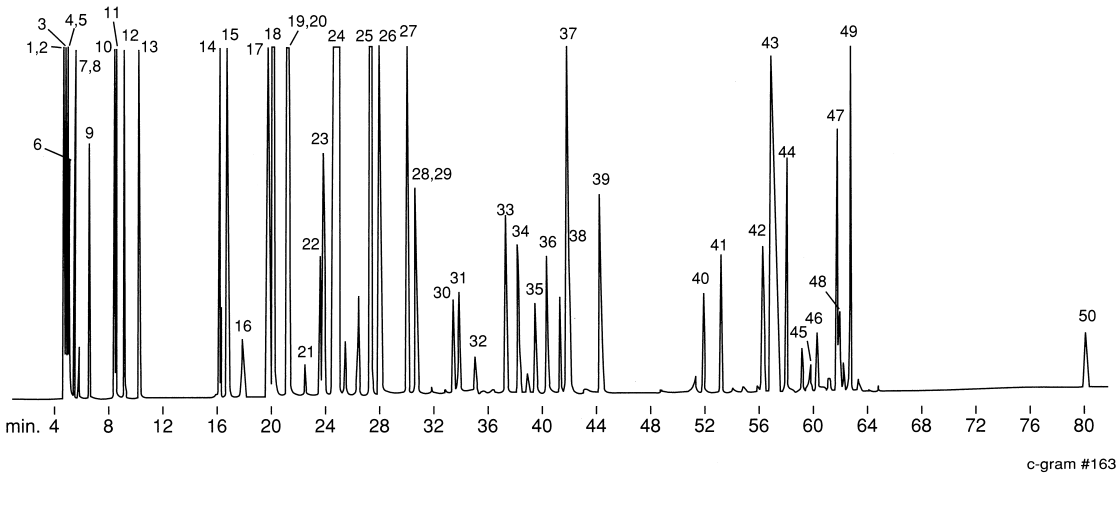


Fig. 3.25.1 Analysis of Standard Fragrance Sample
(Reprinted from RESTEK's 1997 Chromatography Products Guide)

3.25 Analysis of Standard Fragrance Sample (2) - GC

■Analytical Conditions

Column : Rtx-1 (Restek)
60m × 0.53mmI.D. df = 0.5μm
Carrier Gas : He 20cm/sec
Column temperature : 70°C (15min) -2°C/min -190°C
Injection method : Split (1:20), 220°C
Detection : FID, 260°C

■Peaks

1. hexane
2. acetaldehyde
3. acetone
4. methanol
5. ethyl acetate
6. isopropyl alcohol
7. ethanol
8. methylene chloride
9. ethyl propionate
10. α-pinene
11. ethylbutyrate
12. n-hexanal
13. β-pinene
14. sabinene
15. myrcene
16. δ-limonene
17. 1,8-cineole
18. trans-2-hexenal
19. γ-terpinene
20. p-cymene
21. terpinolene
22. octanal
23. nonanal
24. cis-limonene monoxide
25. trans-limonene monoxide
26. furfural
27. citronellal
28. decanal
29. linalool
30. octanol
31. trans-α-bergamotene
32. β-caryophyllene
33. terpinene-4-ol
34. neral
35. α-terpineol
36. neryl acetate
37. valencene
38. geranial
39. carvone
40. geranyl acetate
41. D&L carveol
42. α-ionone
43. D&L carveol
44. β-ionone

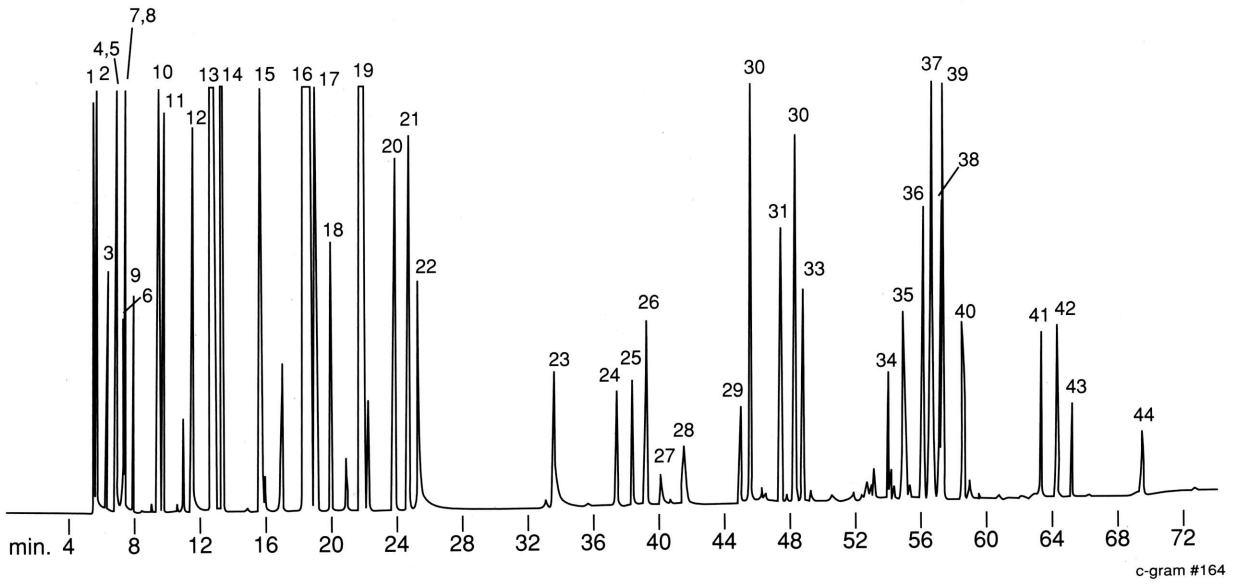


Fig. 3.25.2 Analysis of Standard Fragrance Sample
(Reprinted from RESTEK's 1997 Chromatography Products Guide)

3.26 Essential Oil (Headspace Analysis) - GC

■Explanation

This is an analysis example for essential oil used as flavors for food products.

■Pretreatment

5µL of essential oils were sealed in vials and kept at 40°C for 30 min.

■Analytical Conditions

Column	: CBP1 25m × 0.53mmI.D. df = 3.0µm
Col. Temp.	: 50°C (15min) -5°C/min -200°C
Inj. Temp.	: 230°C
Det. Temp.	: 230°C (FID)
Carrier Gas	: He (10.5mL/min)
Injection method	: Direct Injection
Injection volume	: 0.8mL

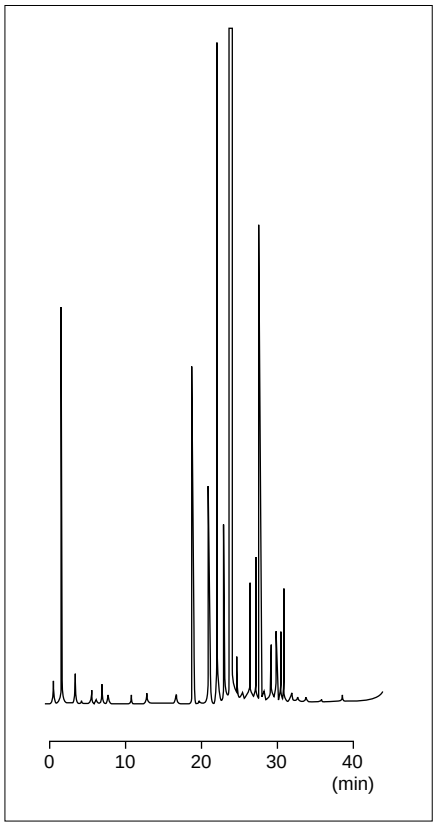


Fig. 3.26.1 Analysis of orange oil

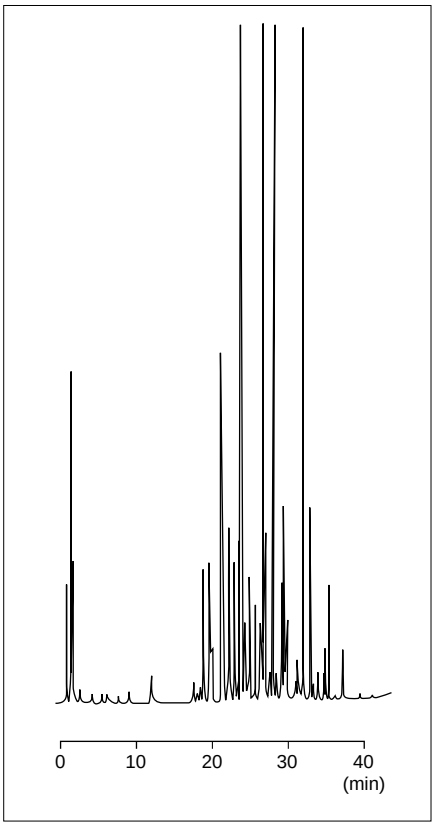


Fig. 3.26.2 Analysis of lavender oil

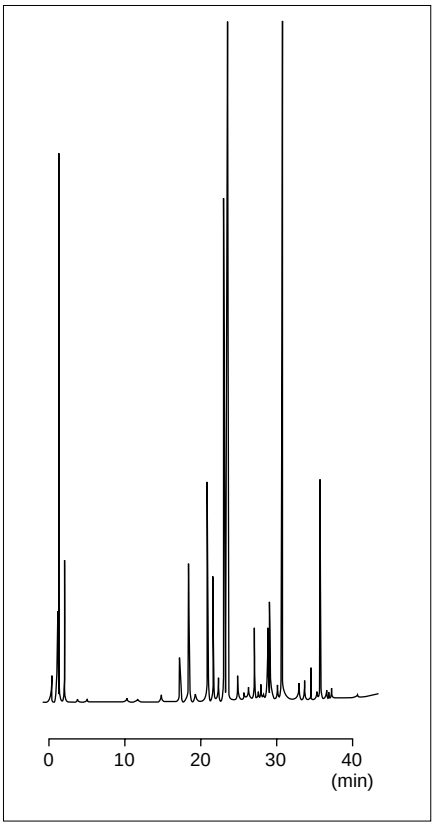


Fig. 3.26.3 Analysis of spearmint oil

3.27 Essential Oil (Direct Analysis) - GC

■Explanation

Here, direct GC analysis examples of peppermint oil and spearmint oil used as flavorings are introduced.

■Analytical Conditions

Column	: ULBON HR-20M 50m × 0.25mmI.D. df = 2.5µm
Col. Temp.	: 60°C -3°C/min -220°C
Inj. Temp.	: 250°C
Det. Temp.	: 250°C (FID)
Carrier Gas	: He (1.4mL/min)
Injection method	: Split (1:15)
Injection volume	: 0.2µL

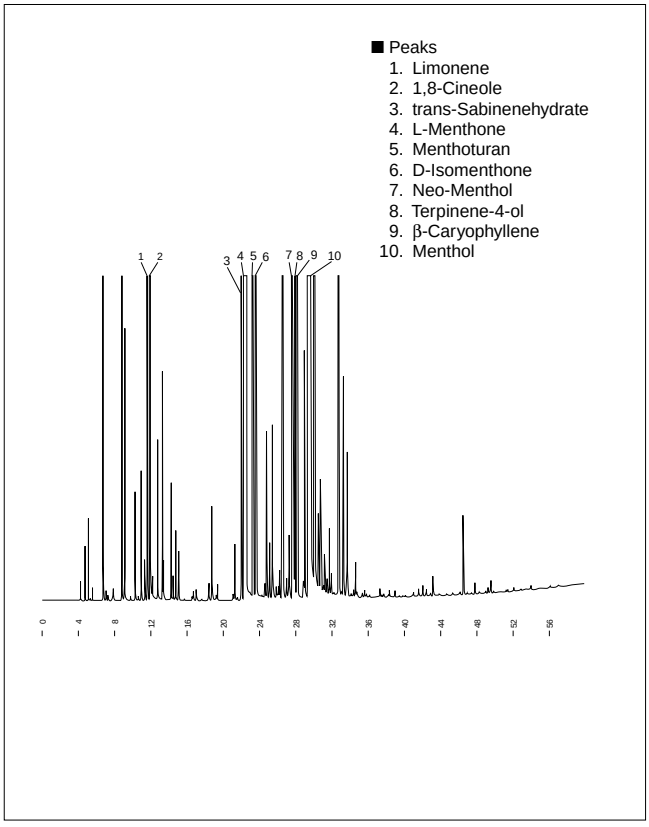


Fig. 3.27.1 Analysis of peppermint oil

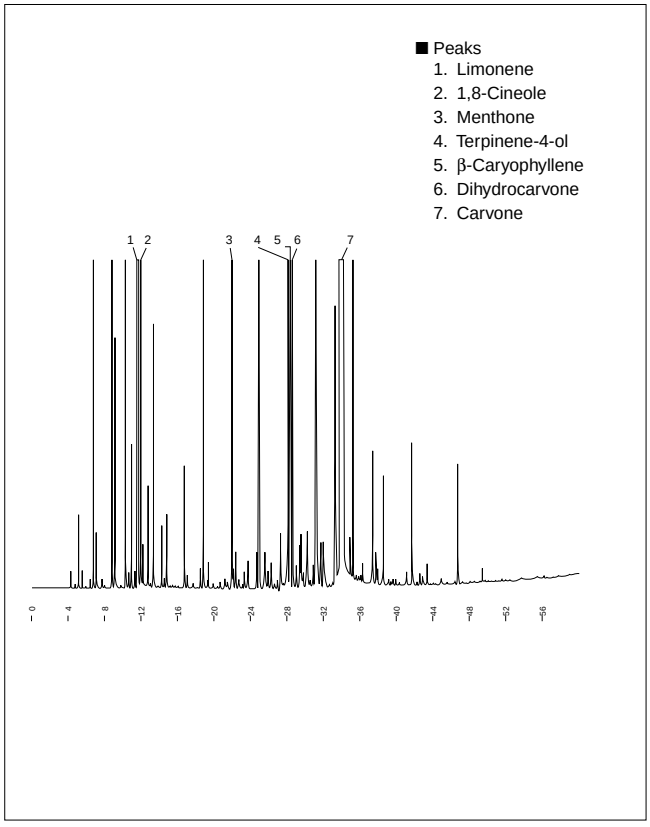


Fig. 3.27.2 Analysis of spearmint oil

3.28 Analysis of Fragrant Material (1) - GC/MS

■Explanation

Many fragrant components are contained in food products. These components are compounds of alcohols, esters, aldehydes, ketones, terpenes and others. The amount and mixture ratio of these components determine the aroma, and any aroma can be artificially synthesized by mixing these components. Here, some 100-aroma components were mixed together and analyzed by GC/MS.

■Analytical Conditions

Instrument	: GCMS-QP5000
Column	: DB-WAX 60m × 0.25mmI.D. df = 0.25µm
Col.Temp.	: 70°C (5min) -3°C/min-210°C(30min)
Inj.Temp.	: 250°C
I/F Temp.	: 230°C
Carrier Gas	: He (180kPa)
Injection	: Split (100:1)

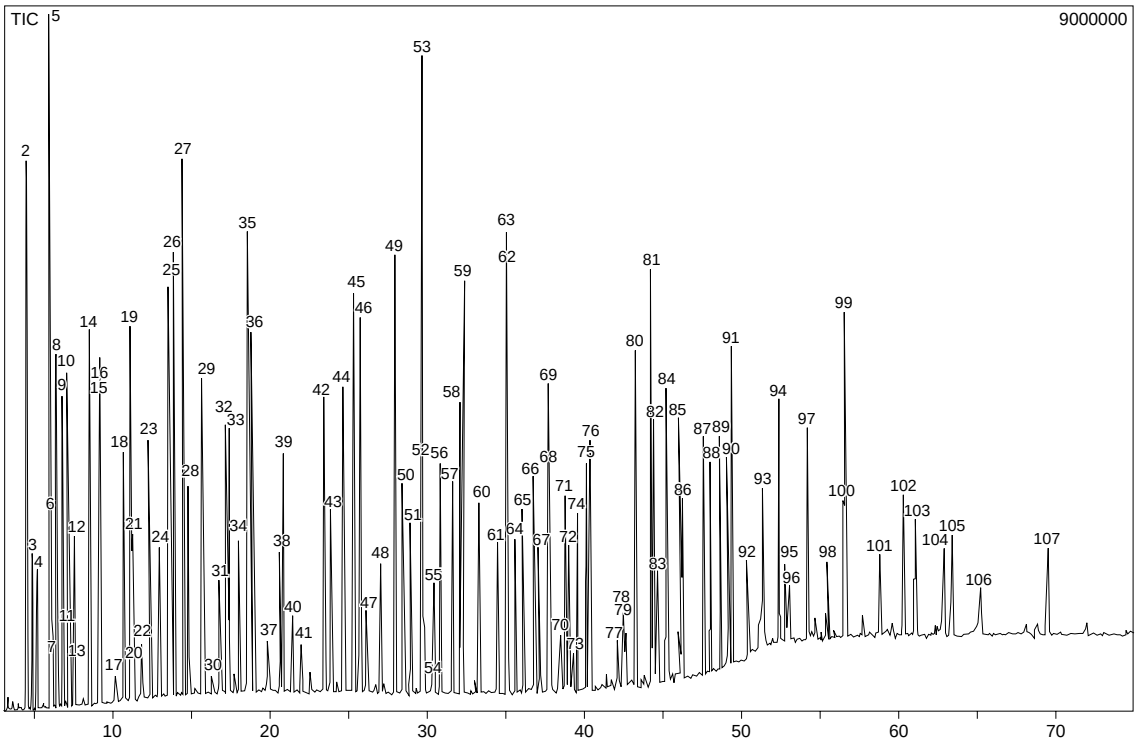


Fig. 3.28.1 TIC chromatogram of fragrant components

3.28 Analysis of Fragrant Material (2) - GC/MS

Table 3.28.1 Component names 1

	Compound	Alcohol	Ester	Aldehyde	Ketone	Terepene	Others
1	Ethyl acetate		○				
2	Diethyl acetal			○			
3	Ethyl alcohol	○					
4	Ethyl propionate		○				
5	i-Butyl acetate		○				
6	Chloroform						○
7							
8	Ethyl n-butyrate		○				
9	Ethyl 2-methyl butyrate		○				
10	Ethyl i-valerate		○				
11	n-Butyl acetate		○				
12	n-Hexanal			○			
13	i-Butyl alcohol	○					
14	n-Amyl acetate		○				
15	n-Butyl alcohol	○					
16	Methyl i-amyl ketone				○		
17							
18	n-Amyl propionate		○				
19	Limonene					○	
20	2-Methyl butyl alcohol	○					
21	n-Amyl furmate		○				
22	c-2-Hexenal			○			
23	Ethyl caproate		○				
24	n-Amyl alcohol	○					
25	i-Amyl n-butyrate		○				
26	n-Hexyl acetate		○				
27	Methyl n-hexyl ketone				○		
28	i-Amyl i-valerate		○				
29							
30							
31	Ethyl lactate		○				
32	n-Hexanol	○					
33	Ethyl n-hexyl ketone				○		
34	Allyl caproate		○				
35							
36	Methyl n-heptyl ketone				○		
37	t-3-Hexenol	○					
38							
39	Ethyl caprylate		○				
40	Acetic acid						○
41	Furfural			○			
42	Methyl n-octyl ketone				○		
43	Tetrahydro furfuryl alcohol	○					
44	Benzaldehyde			○			
45	Ethyl nonanoate		○				
46	Linalool					○	
47							
48	Diethyl malonate		○				
49	Methyl n-nonyl ketone				○		
50	Ethytl levulinate		○				
51	Methyl benzoate		○				
52	Ehtyl caprate		○				
53	l-Menthol					○	
54							

3.28 Analysis of Fragrant Material (3) - GC/MS

Table 3.28.2 Component names 2

	Compound	Alcohol	Ester	Aldehyde	Ketone	Terepene	Others
55	Furfuryl alcohol	○					
56	Ethyl benzoate		○				
57	Phenyl diethyl acetate		○				
58							
59	Methyl n-decyl ketone				○		
60	Benzyl acetate		○				
61	Methyl phenyl acetate		○				
62	Dimethyl benzyl carbinyl acetate		○				
63	Allyl caprate		○				
64	Ethyl phenyl acetate		○				
65	Allyl β-cyclohexyl propionate		○				
66	Phenethyl acetate		○				
67	Anethol					○	
68	Caproic acid						○
69	Ethyl laurate		○				
70	t-2-Decenal			○			
71	Benzyl n-butyrate		○				
72	Benzyl alcohol	○					
73	Phenetyl propionate		○				
74	i-Butyl phenyl acetate		○				
75	Dimethyl benzyl carbinylbutyrate		○				
76	Phenyl ethyl alcohol	○					
77							
78							
79	Phenyl ethyl propionate		○				
80	Phenethyl i-valerate		○				
81	Methyl n-tridecyl ketone				○		
82	Anisaldehyde			○			
83	γ-Nonalactone						○
84	Ethyl myristate		○				
85	Triacetine						○
86	Methyl cinnamate		○				
87	Benzylidene acetone				○		
88	Ethyl cinnamate		○				
89	γ-Decalactone						○
90	Eugenol					○	
91	Phenethyl caproate		○				
92	δ-Decalactone						○
93	Heliotropine						○
94	γ-Undecalactone						○
95	Anisalcohol	○					
96	Cinnamy alcohol	○					
97	Diethyl sebacate		○				
98							
99	γ-Dodecalactone						
100	Phenethyl octanoate		○				
101	δ-Dodecalactone						○
102	TEC						○
103	Benzophenone				○		
104	Ethyl vanillin						○
105							
106	vanilline						○
107	Benzyl benzoate		○				

3.29 Analysis of Potassium Bromate Contained in Bread - LC

■Explanation

The use of potassium bromate as an additive is allowed in bread production to make the bread-making process more effective. However, to ensure safety, it must not remain in the final product. Therefore, it is necessary to verify that there is no potassium bromate left in the bread. Chemical Hygiene No. 119 issued from the Japanese Ministry of Health, Labour and Welfare on September 11, 1997 stipulates the post-column derivatization HPLC method using o-dianisidine as a reaction reagent to analyze potassium bromate in bread.

This section shows an example of analyzing potassium bromate contained in bread.

Fig. 3.29.1 shows the chromatogram obtained by analyzing commercially sold bread after the pretreatment procedure shown in Fig. 3.29.2 that conforms to Chemical Hygiene No. 119 (lower chromatogram), and the chromatogram obtained when 326 µg of potassium bromate standard (equivalent to 250 µg of bromate ions) was added to 10 grams of bread before pretreatment (upper chromatogram). No bromate ions were detected when analyzing bread alone. When analyzing the bread with potassium bromate added, 325.4 µg of potassium bromate (249.4 µg of bromate ions) was quantified in 10 grams of bread, demonstrating approximately 100% recovery rate.

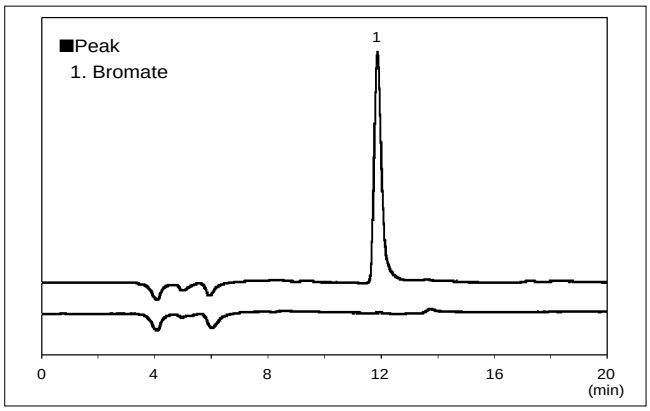


Fig. 3.29.1 Analysis of Potassium Bromate in Bread

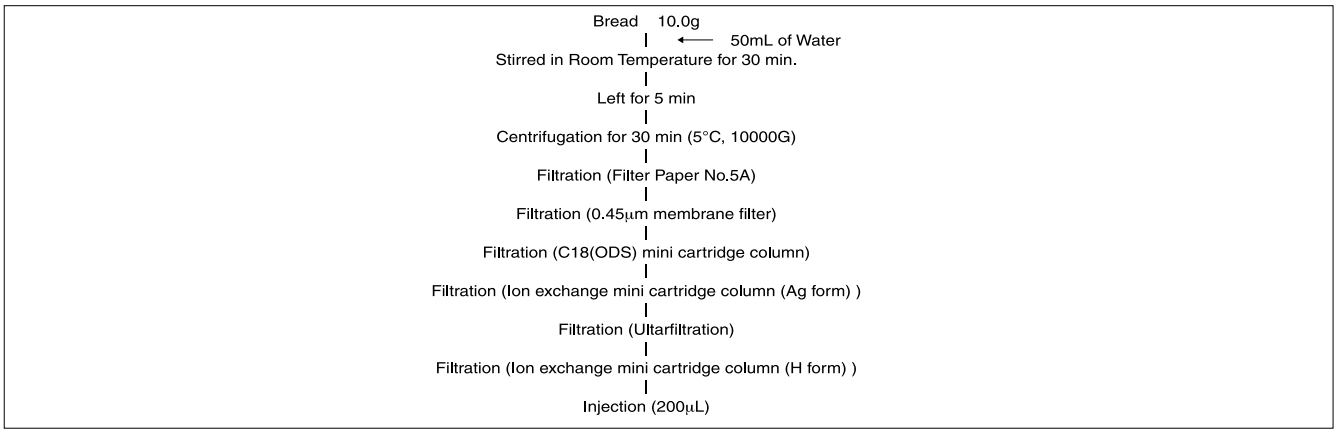


Fig. 3.29.2 Pretreatment Procedure

Table 3.29.1 Analytical Conditions

Column	:	Shim-pack VP-ODS (250mmL. × 4.6mm I.D.)
Mobile Phase	:	100mL of methanol, 2.0g of acetic acid and 19g of tetrabutylammonium hydroxide were added to 700mL of Water, and pH of solution was adjusted to 6.3 - 6.5. And then, this solution was diluted to 1000mL with Water.
Flow rate	:	1.0mL/min
Temperature	:	40°C
Injection Volume	:	200µL
Reaction regent	:	A ; 60mL of nitric acid (70%), 10.0g of potassium bromide was added to 700mL of Water. B ; 500mg of o-dianisidine dihydrochloride was added to 200mL of methanol. Solution A and B were mixed, and diluted to 1000mL with Water.
Reaction unit	:	Piping Kit for Bromate Analysis
Temperature	:	60°C
Detection	:	Absorption (450nm)

3.30 Ethylene Glycols in Wine - GC

■Explanation

Normally wine does not contain ethylene glycol but there have been reports of temporary errors where diethylene glycol was mixed into wine.
Here, ethylene glycol and diethylene glycol have been added to wine and directly analyzed by GC. Analysis was possible without any interference from impurities in the wine.

References

Shimadzu Application News No. G110

■Pretreatment

Ethylene glycol and diethylene glycol were added to a shop-sold wine for direct analysis.

■Analytical Conditions

Column	: ULBON HR-20M 25m × 0.25mmI.D. df = 0.25µm
Col. Temp.	: 150°C
Inj. Temp.	: 200°C
Det. Temp.	: 200°C (FID)
Carrier Gas	: He 2mL/min
Injection	: Split 1:30

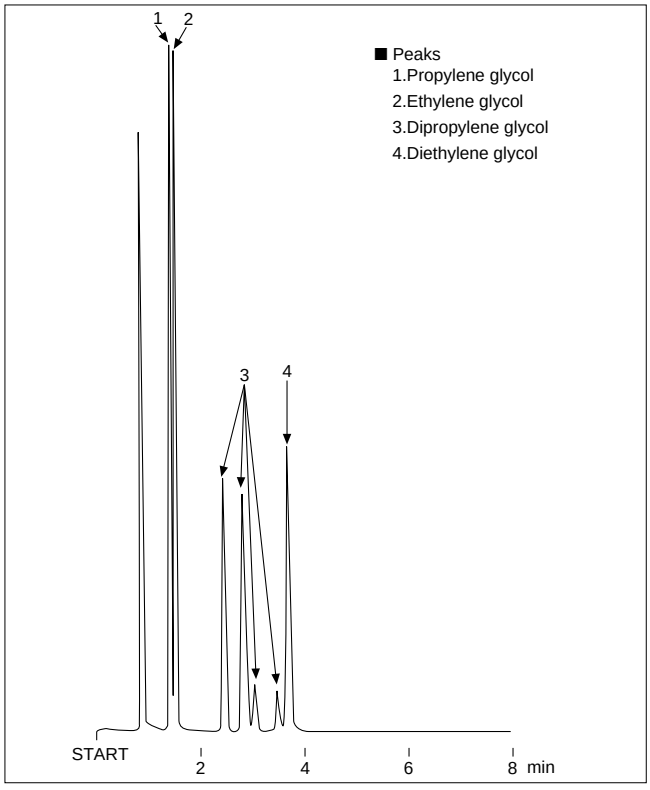


Fig. 3.30.1 Analysis of glycols (standard products)

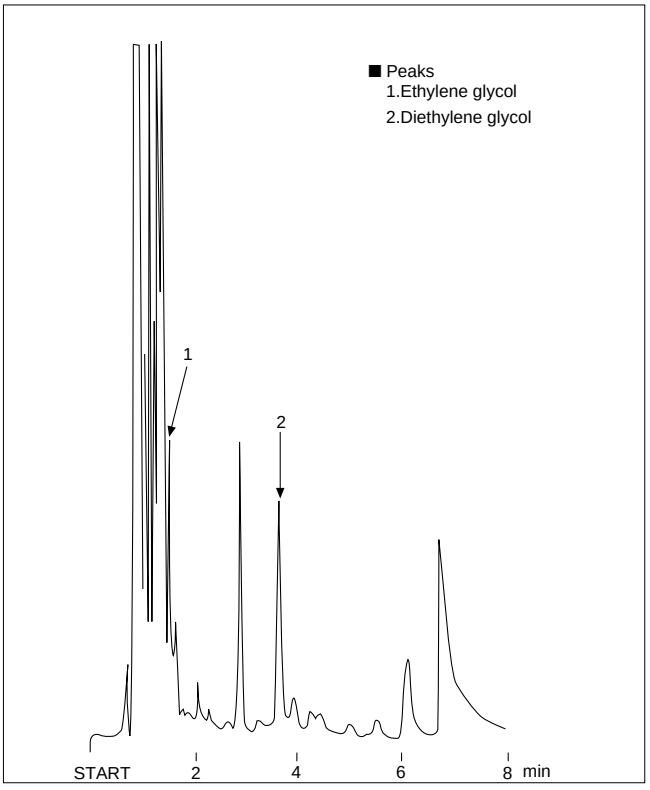


Fig. 3.30.2 Analysis of shop-sold wine with glycols added

3.31 Analysis of Propylene Glycol and Other Substances - GC

■Analytical Conditions

Column : CBP20-M25-025 (Shimadzu)
25m × 0.22mmI.D. df = 0.25μm
Carrier Gas : He 2mL/min
Column temperature : 50°C -3°C/min -150°C
Injection method : Split, 200°C
Detection : FID, 200°C

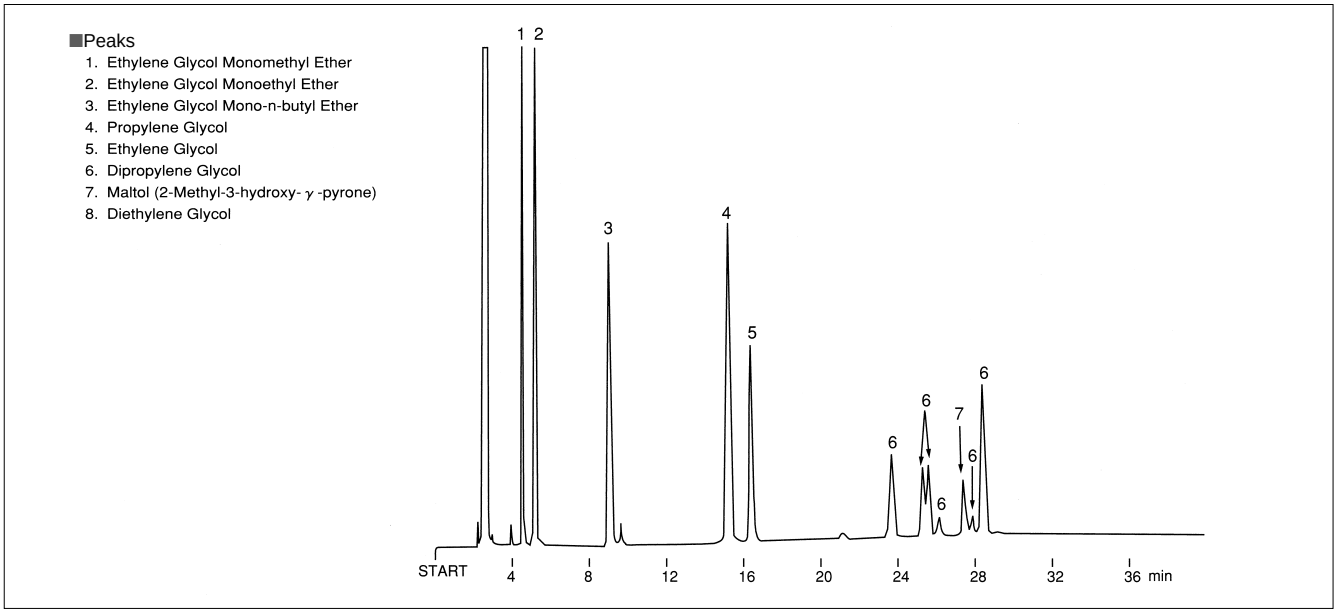


Fig. 3.31.1 Analysis of Propylene Glycol and Other Substances

■Analytical Conditions

Column : Thermon-3000 5% on SHINCARBON (Shinwa Chemical Industries)
1.6m × 3.2mmI.D.
Carrier Gas : N_2 40mL/min
Column temperature : 170°C
Injection inlet temperature : 260°C
Detection : FID, 260°C

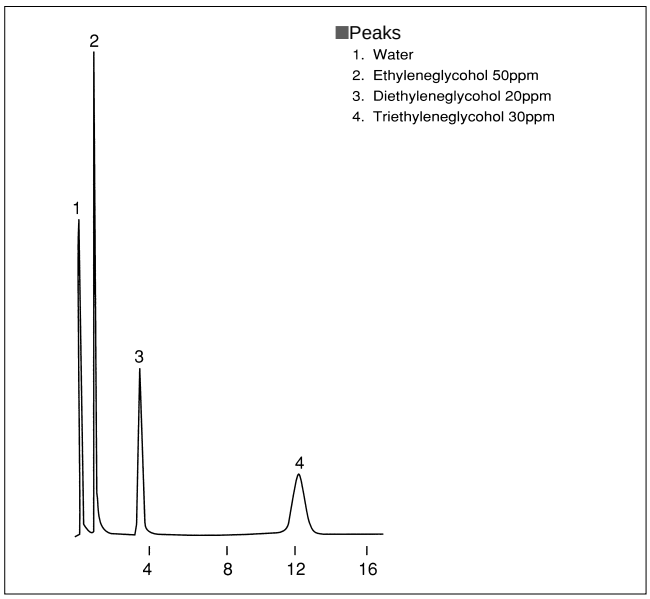


Fig. 3.31.2 Analysis of Ethylene Glycols

4.1 Analysis of Shellfish Toxins (1) - LC

■Explanation

In recent years shellfish poisoned with paralytic shellfish toxins are found in various regions, causing major damage to the marine product industry and serious problems in food hygiene.

Paralytic shellfish toxin is a neurotoxin produced by a phytoplankton dinoflagellate, and is known by the component names such as saxitoxin or gonyautoxin.

Post column derivatization fluorescent detection LC analysis method was used by Nagashima and Oshima to analyze this shellfish toxin.

Here, this method is used in an analysis example of gonyautoxin (GTX) 1-4 standard sample.

References

LC talk, No. 36 from Shimadzu Corporation (1995)
Y.Nagashima, et.al.,
Nippon Suisan Gakkai, 53 (5), 819 (1978).
Y.Oshima, et.al., "Mycotoxins and Phycotoxins '88",
Elsevier Science Publishers, New York, 1989, 319.

■Analytical Conditions

- Separation conditions

Column	: STR ODS-II (150mmL. × 4.0mmI.D.)
Mobile phase	: 10mM (Sodium) Phosphate Buffer containing 4mM (Sodium) Heptanesulfonate (pH 7.0)
Temperature	: 40°C
Flow rate	: 0.8mL/min

- Reaction Conditions

Primary reaction reagent	: 50mM (Sodium) Borate Buffer containing 5mM Periodic Acid (pH 9.5)
Flow rate	: 0.4mL/min
Temperature	: 60°C
Secondary reaction reagent	: 110mM Phosphate Buffer (pH 2.1)
Flow rate	: 0.4mL/min
Temperature	: 40°C
- Detection	: Fluorescence (Ex330nm Em390nm)

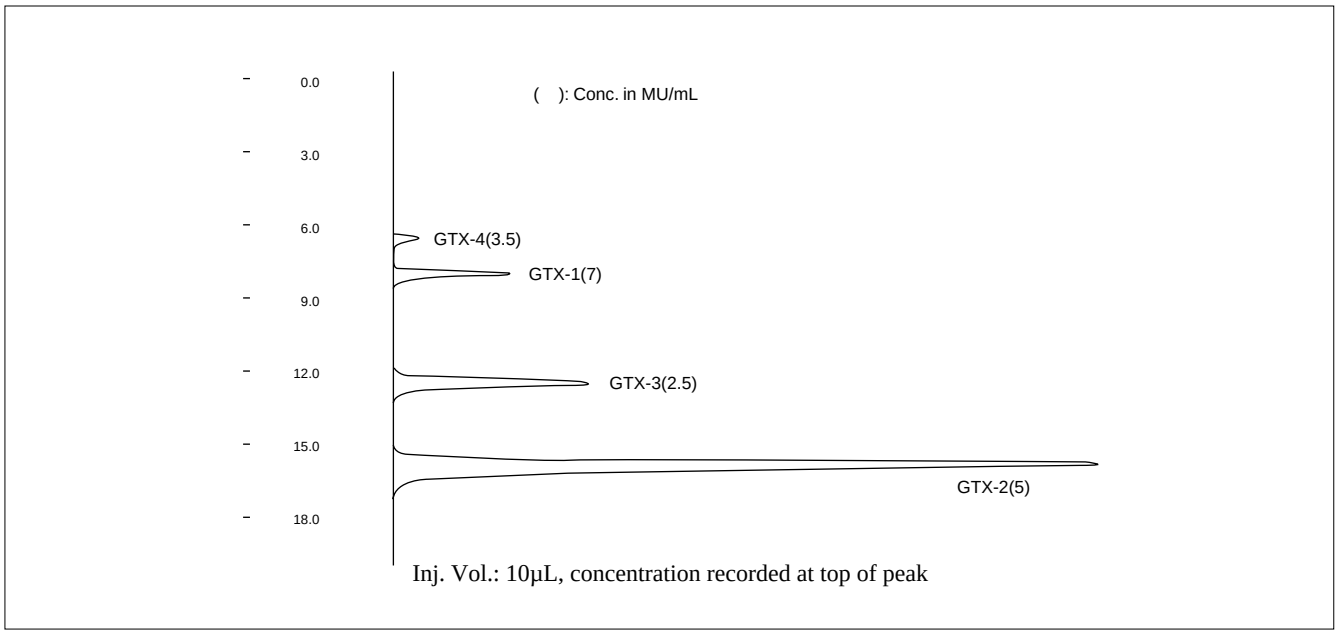
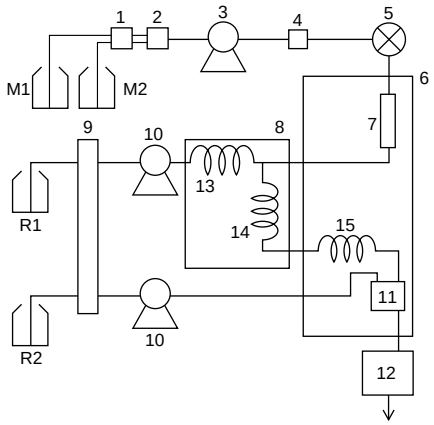


Fig. 4.1.1 Analysis example of gonyautoxin (GTX) 1-4 standard sample

4.1 Analysis of Shellfish Toxins (2) - LC

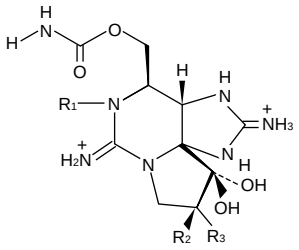


1. 9. Degassing units
2. Low-pressure gradient unit
3. 10. Solvent delivery units
4. Mixer
5. Auto injector
6. Column oven
7. Analysis column
8. Chemical reaction tank
11. Blender
12. Fluorescent detector
13. 14. 15. Reaction coils

M1, M2 Mobile phases

R1, R2 Reaction liquids

Fig. 4.1.2 Flow line



R1	R2	R3	
H	H	H	STX
OH	H	H	neoSTX
OH	H	OSO_3^-	GTX1
H	H	OSO_3^-	GTX2
H	OSO_3^-	H	GTX3
OH	OSO_3^-	H	GTX4

Fig. 4.1.3 Structural formula of shell toxin components

4.2 Analysis of Fumonisin in Sweet Corn (1) - LC

■Explanation

The mycotoxin family member fumonisin is related to fusarium branch and is known to be the cause of equine leukoencephalomalacia and lung edema in pigs. Recent research also points to its involvement in human esophageal cancer. Here, this component was analyzed using pre-label fluorescent derivatization and detection incorporating OPA agent.

References

G.S.Shepland,et.al.,J.Liquid Chromatogr.,13,2077 (1990)

■Pretreatment

200µL of thiol agent and 200µL of OPA agent was added to 100µL of sample solution. After mixed and left to stand for 3 min, 10µL of the mixture was injected to HPLC.

Thiol agent: 0.1M (sodium) borate buffer (pH 9.2) containing 50mM 3-mercaptopropionic acid
OPA agent: A/B = 1/4 mixture
A: 0.25M o-Phthalaldehyde methanol solution
B: 0.1M (sodium) borate buffer (pH 9.2)

■Analytical Conditions

Column	: STR ODS-II (150mmL. × 4.6mm I.D.)
Mobile phase	: Methanol/50mM Citrate Buffer (pH 4.3) (7/3, v/v)
Temperature	: 40°C
Flow rate	: 1.0mL/min
Detection	: Fluorescence Ex335nm Em440nm

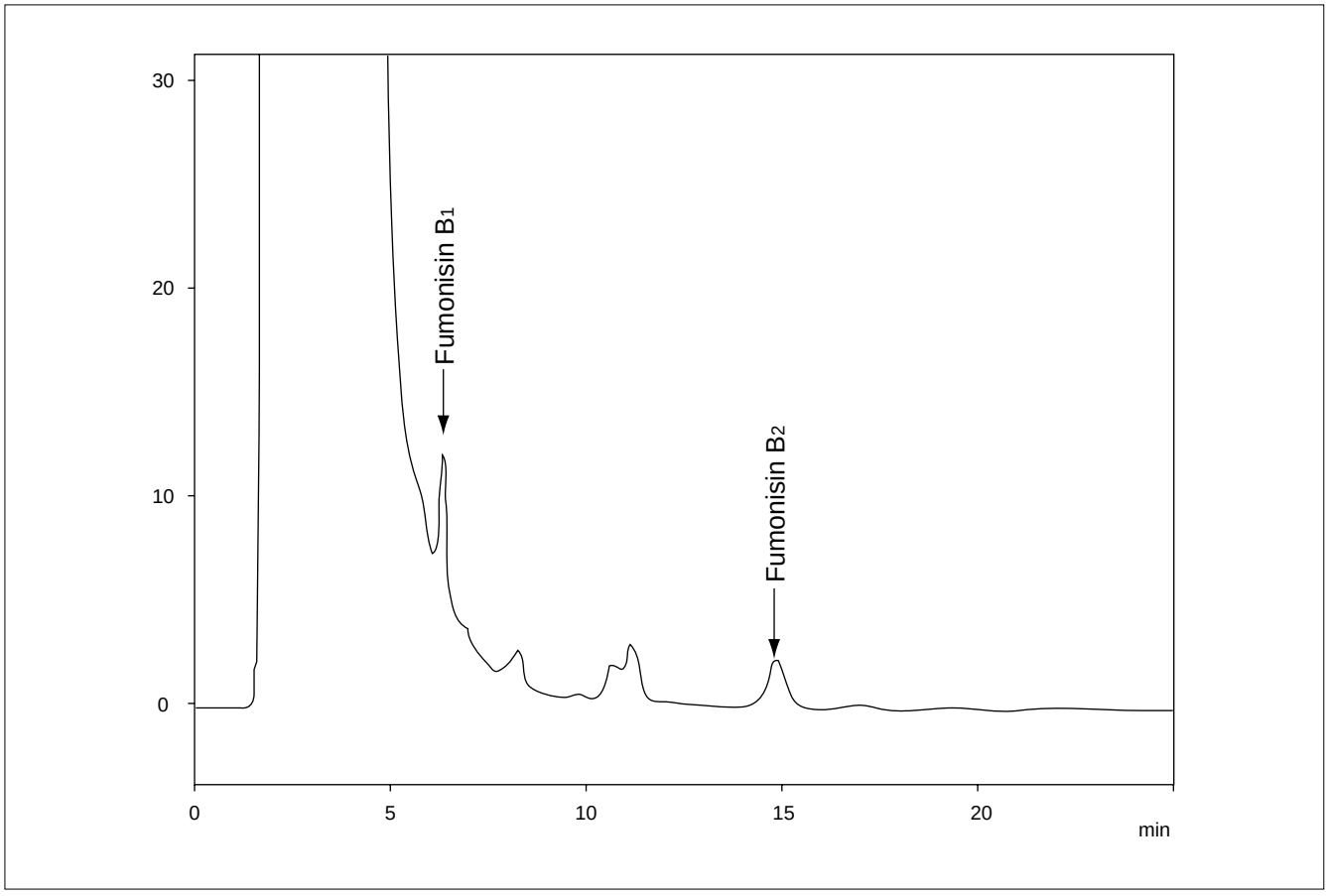


Fig 4.2.1 Analysis example of fumonisin in sweet corn

4.2 Analysis of Fumonisin in Sweet Corn (2) - LC

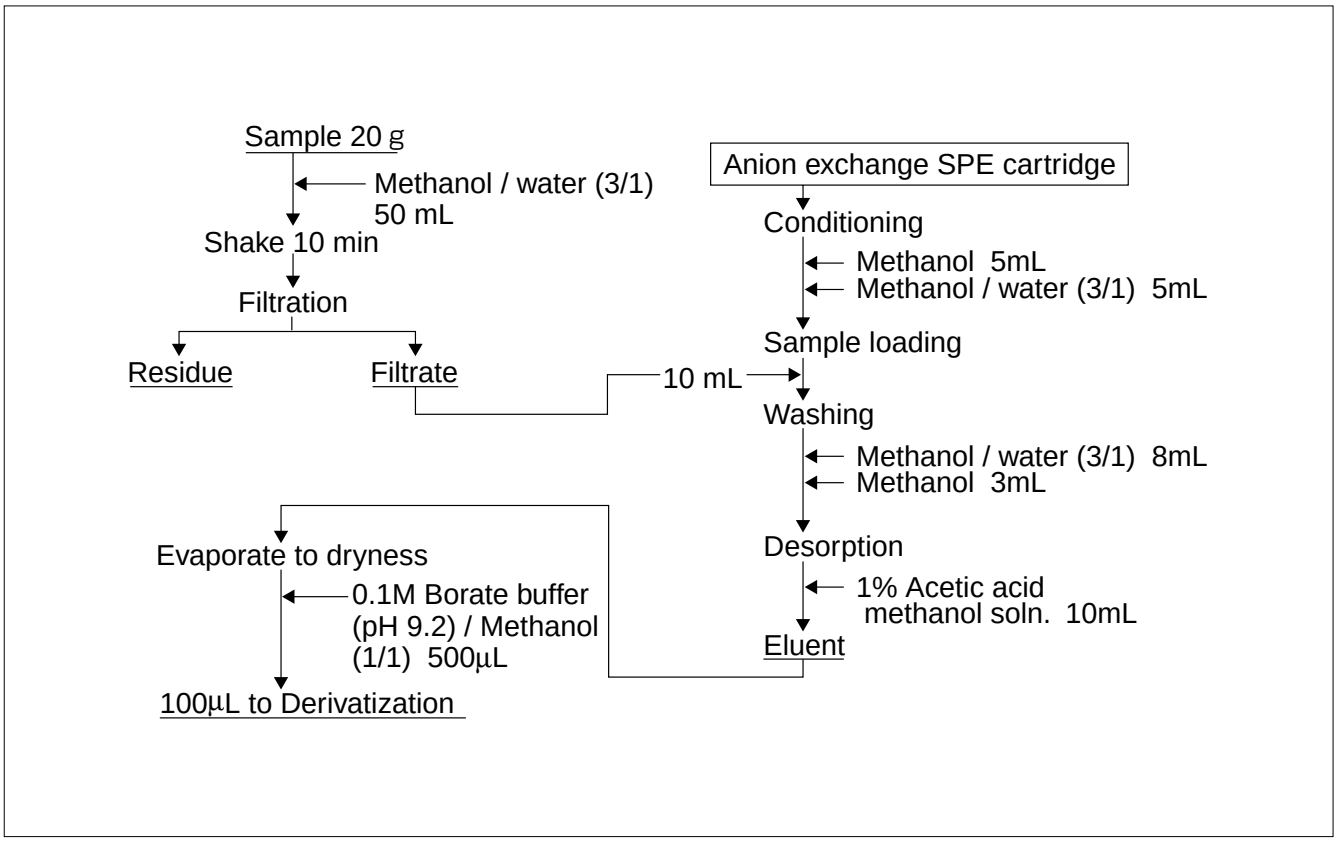


Fig. 4.2.2 Pretreatment flowchart for sweet corn

4.3 Analysis of Aflatoxins (1) - LC

■Explanation

Aflatoxins are toxins produced by the Aspergillus family of molds that grow in tropical and subtropical regions. Aflatoxins indicate a powerful acute toxicity (having a degenerative effect on the brain, liver and kidneys, etc.) and trace amounts of aflatoxins can cause liver cancer if absorbed over a long period of time. Aflatoxins include the structurally similar B_1, B_2, G_1 and G_2 types and their metabolites M_1 and M_2, of which the most toxic with respect to animals is B_1. The Japanese Food Sanitation Law stipulates that the B_1 aflatoxin exceeding 10 ppb must not be detected in any food products. This section shows an example of analyzing an actual sample.

■Analytical Conditions

Column	: Silica gel (5.5 µm particle diameter) (100 mm L. × 4.0 mm I.D.)
Mobile phase	: Toluene/Ethylacetate/Formic Acid/Methanol = 90/5/2.5/2.5 (v/v)
Temperature	: 40°C
Flow rate	: 1.1mL/min
Detection	: Fluorescence Ex 365nm Em 425nm

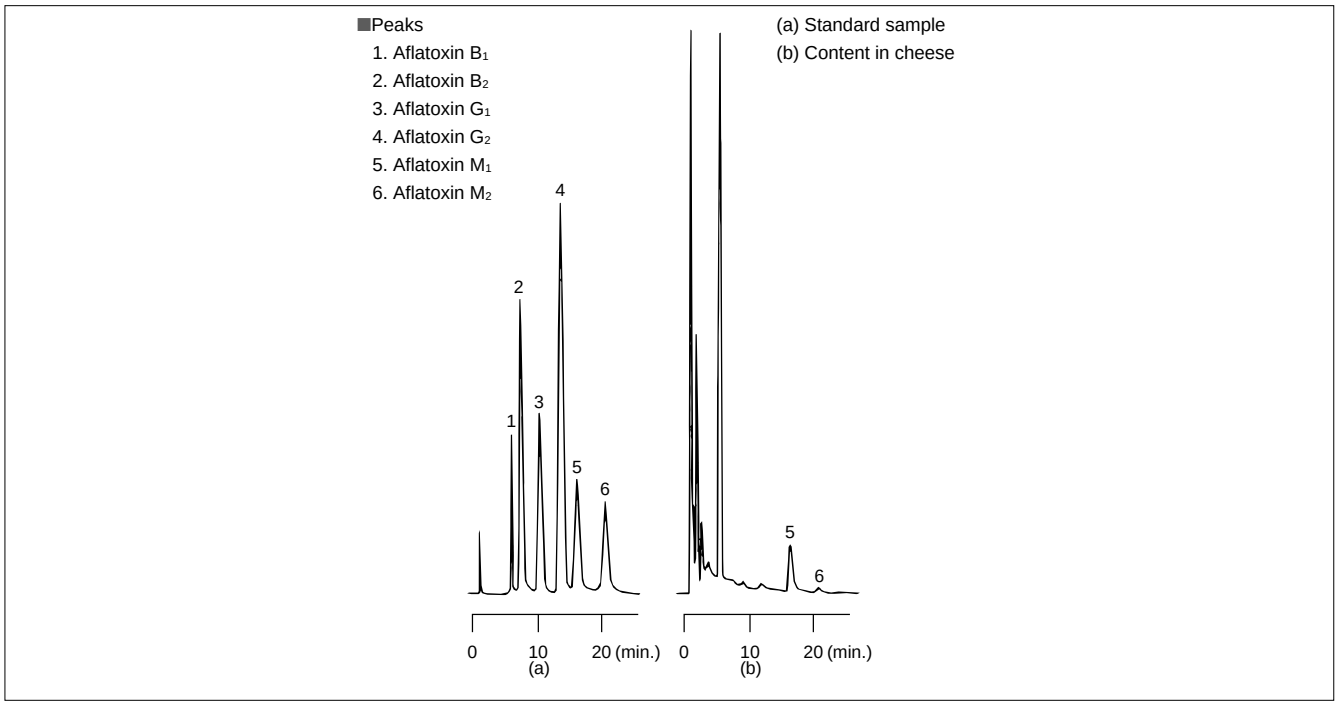


Fig. 4.3.1 Analysis of Aflatoxins Contained in Cheese

4.3 Analysis of Aflatoxins (2) - LC

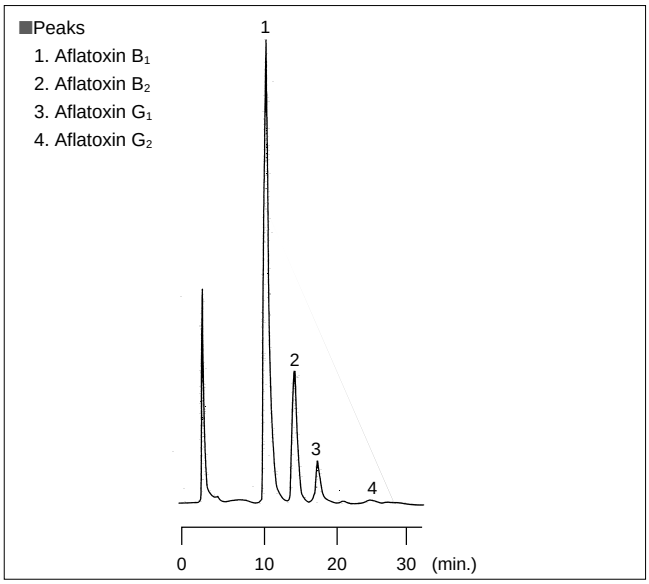


Fig. 4.3.2 Analysis of Aflatoxins Contained in Peanuts

■Analytical Conditions

Column	: Zorbax SIL (250mmL. × 2.1mmI.D.)
Mobile phase	: Toluene / Ethyl Acetate / Formic Acid / Methanol = 890 / 75 / 20 / 15 (v/v)
Temperature	: 40°C
Flow rate	: 0.37mL/min
Detection	: Fluorescence (Ex 365nm, Em 425nm)

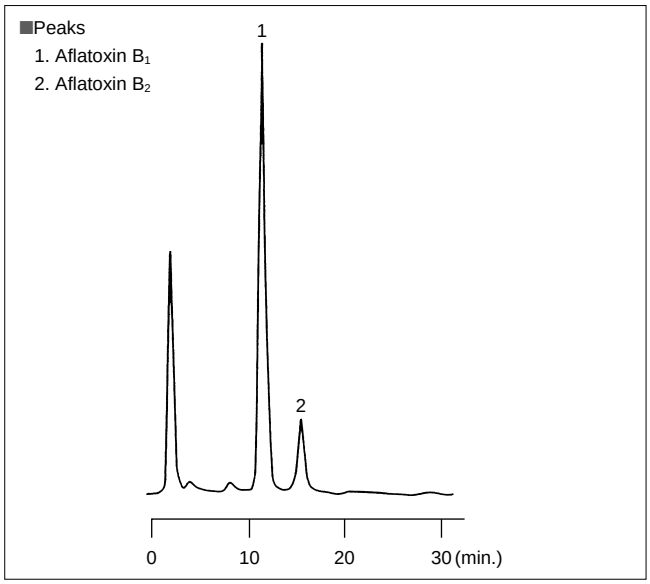


Fig. 4.3.3 Analysis of Aflatoxins Contained in Corn

■Analytical Conditions

Column	: Zorbax SIL (250mmL. × 2.1mmI.D.)
Mobile phase	: Toluene / Ethyl Acetate / Formic Acid / Methanol = 890 / 75 / 20 / 15 (v/v)
Temperature	: 40°C
Flow rate	: 0.37mL/min
Detection	: Fluorescence (Ex 365nm, Em 425nm)

4.3 Analysis of Aflatoxins (3) - LC

Pretreatment Procedure for the Analysis of Aflatoxins in Cheese

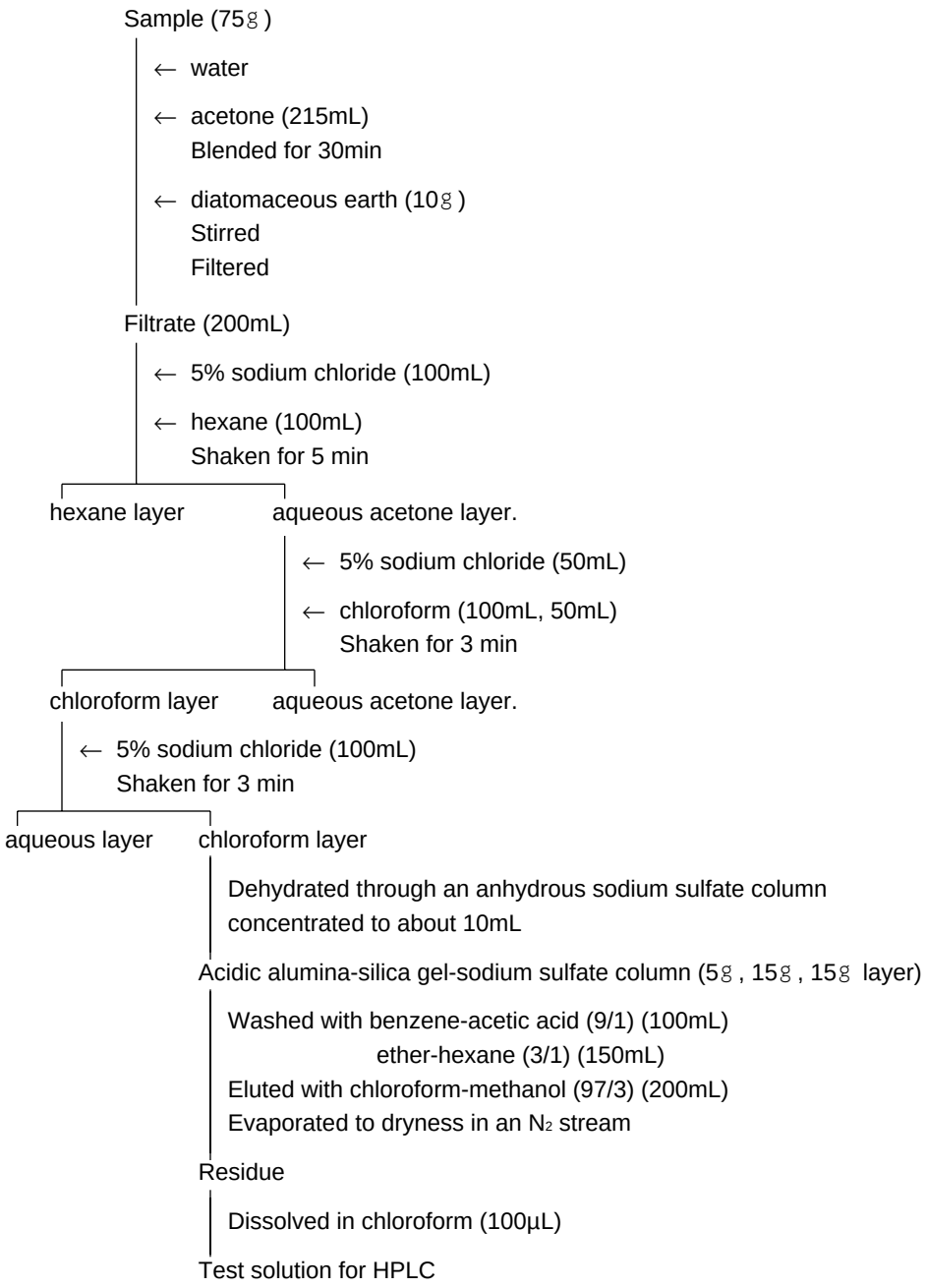


Fig. 4.3.4 Extraction of Aflatoxins

4.4 Analysis of Aflatoxins Using LC/MS (1) - LC/MS

■Explanation

Over 300 mold toxins, including aflatoxin, patulin and fumonisin, have been identified. HPLC and LC/MS are useful for analyzing these toxins. LC/MS with outstanding qualitative capabilities is especially useful for analyzing multiple mold toxins, particularly aflatoxins. The Japanese MHLW notice on March 26, 2002 stipulates LC and LC/MS as the analytical methods for aflatoxins in grains, legumes, seeds and nuts, and spices.

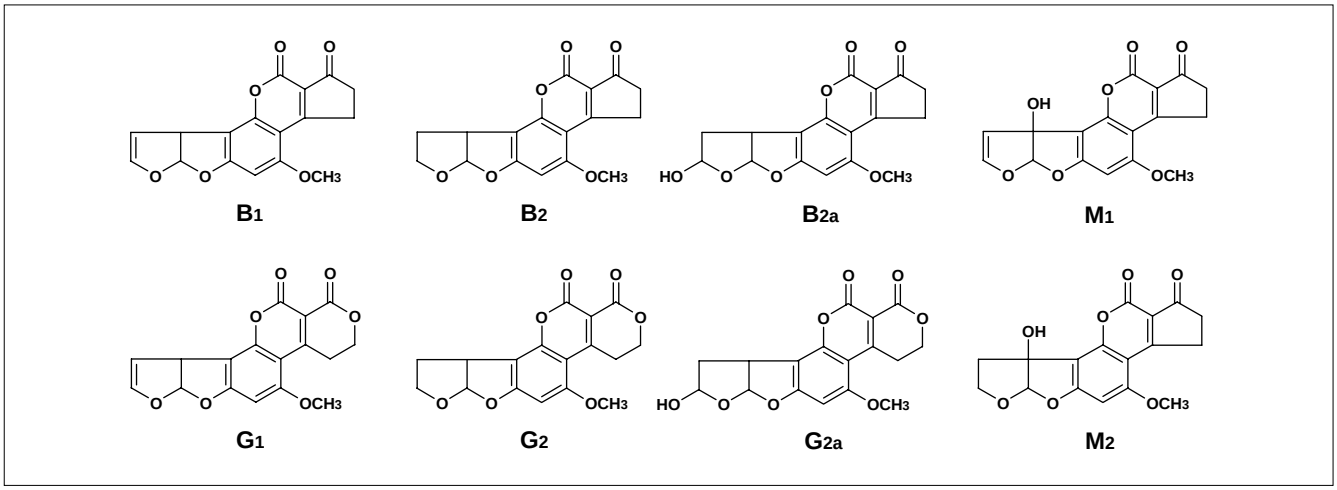


Fig. 4.4.1 Structures of Aflatoxins

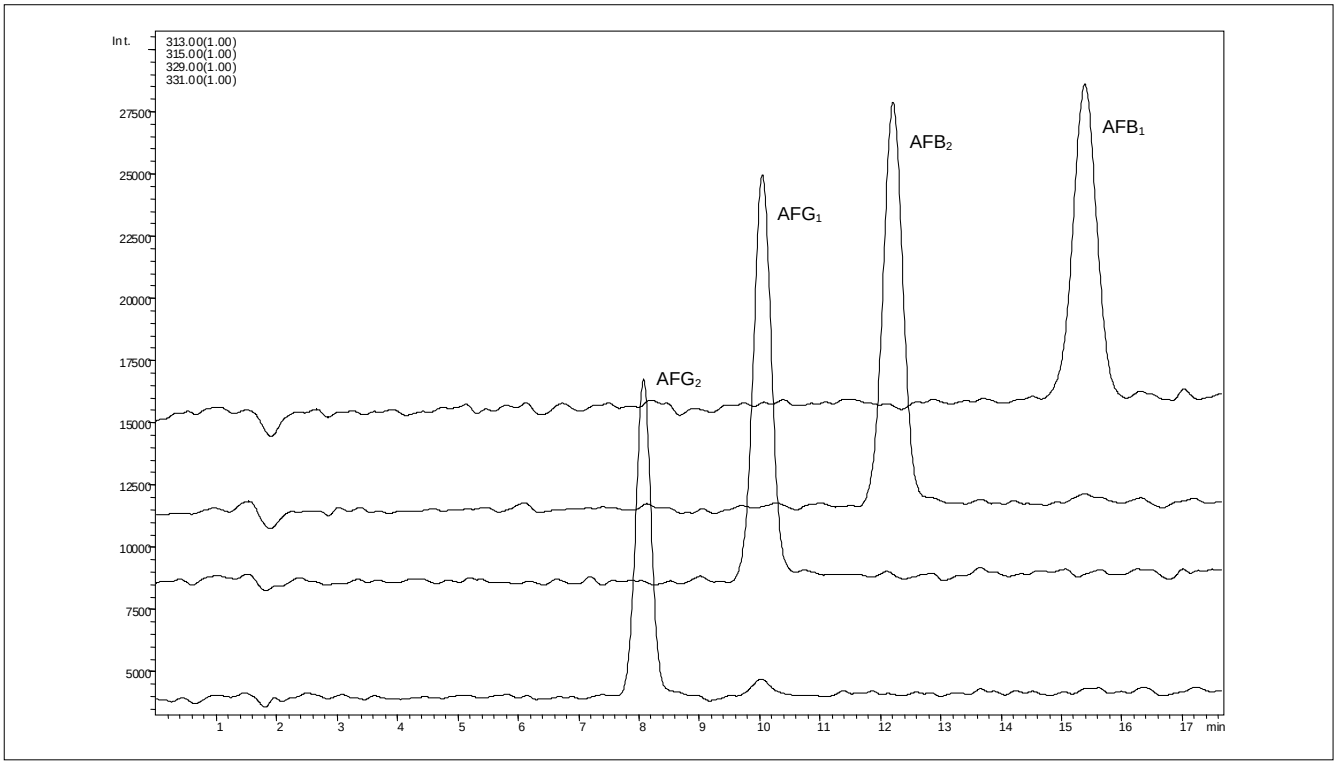


Fig. 4.4.2 SIM Chromatogram of Four Varieties of Aflatoxin
(6 µL injected at 2.5 ng/mL. Analytical conditions conform to the MHLW notice.)

4.4 Analysis of Aflatoxins Using LC/MS (2) - LC/MS

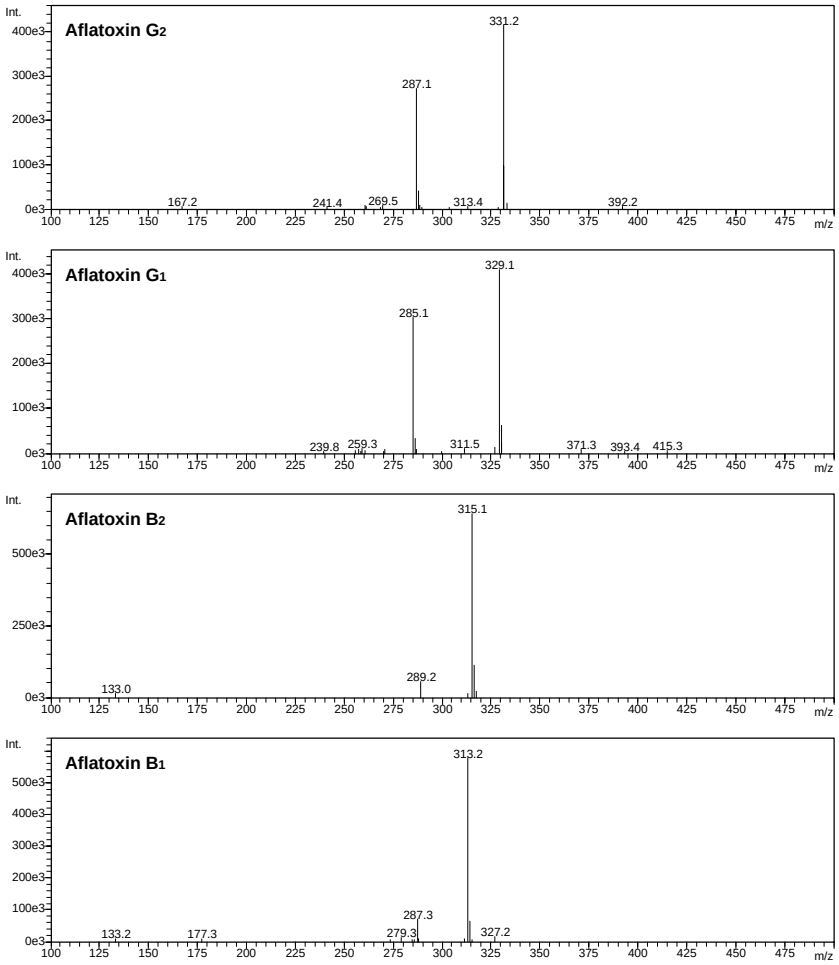


Fig. 4.4.3 Mass Spectra for Aflatoxin G2, G1, B2 and B1

Table 4.4.1 Analytical Conditions

Instrument	:	LCMS-2010A
Column	:	Shim-pack VP-ODS(150mmL. × 2.0mmI.D.)
Mobile phase	:	Acetonitrile / Methanol / 10 mM Ammonium Acetate Aqueous Solution = 2/6/15 (v/v/v)
Flow rate	:	0.2 mL/min
Column temperature	:	40°C
Sample injection volume	:	6μL
Probe voltage	:	+4.5kV(ESI-positive mode)
Nebulizer gas flow rate	:	2.5L/min
CDL voltage	:	0 V
CDL temperature	:	300°C
Block heater temperature	:	250°C
Q-array voltage	:	Scan mode
Drying gas flow rate	:	0.1MPa
Analysis mode	:	SIM (m/z 313, 315, 329 and 331)

4.5 Analysis of Patulin Using LC/MS - LC/MS

■Explanation

Patulin is a mycotoxin produced by Penicillium and Aspergillus molds and is detected in fruit to which mold has adhered. Some of the standards for food products and additives were revised in accordance with Notification No. 369 issued by Japan's Ministry of Health, Labour and Welfare (July 2003). New qualitative and quantitative tests using an LC-UV detector and confirmation tests using LC/MS were established as test methods for patulin. It is specified that the residual patulin content in products whose basic ingredient is apple juice or apple juice concentrate must not exceed 0.050 ppm.

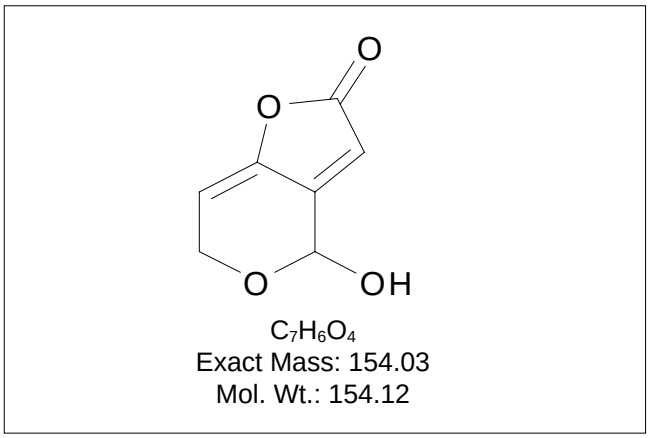


Fig. 4.5.1 Structure of patulin

■Analytical Conditions

Instrument	: Shimadzu LCMS-2010A
Column	: Shim-pack VP-ODS (150 mmL. x 2.0 mmI.D.)
Mobile phase	: Water/Acetonitrile = 96/4 (v/v)
Flow rate	: 0.2 mL/min
Temperature	: 40ºC
Detector	: SPD-M10A (276 nm)
Sample storage temperature	: 5ºC
Sample injection volume	: 50 µL
Ionization mode	: APCI-Positive
Probe voltage	: 4.5 kV
Nebulizer-gas flow rate	: 2.5 L/min
Drying-gas pressure	: 0.05 MPa
CDL temperature	: 250ºC
Block heater temperature	: 200ºC
CDL voltage	: S-Mode
Q-array voltage	: S-Mode
Selected ion mass number	: m/z 154.95 $(M+H)^+$(patulin)

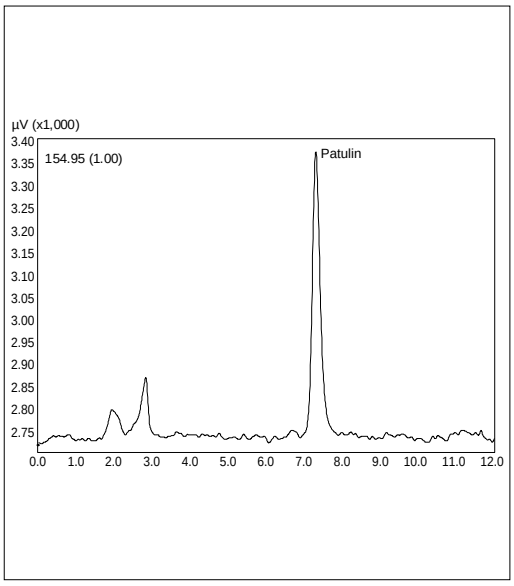


Fig. 4.5.2 Chromatogram for standard patulin sample (50 ppb, 50 µL)

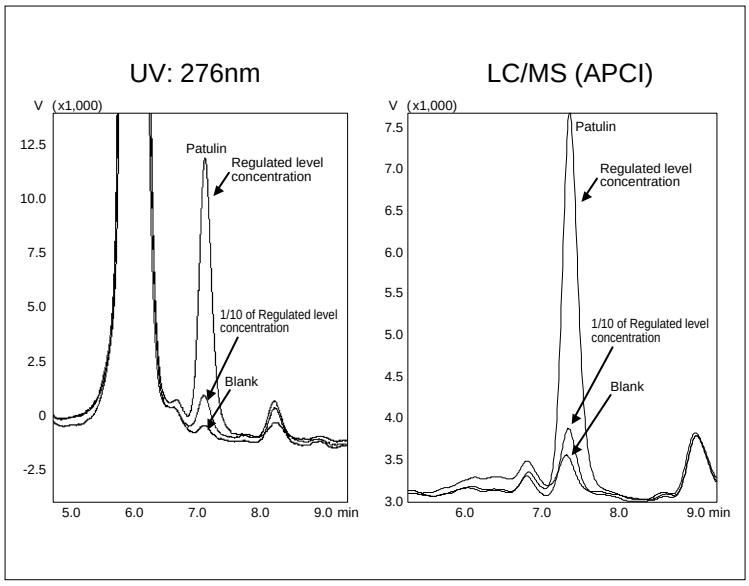


Fig. 4.5.3 Chromatogram for patulin-added sample

4.6 Analysis of Organotin Compounds Using Capillary GC-FPD - GC

■Explanation

Tributyltin (TBT) and triphenyltin (TPT) are widely used as antifouling coatings for ships and fishing nets. Although the production of coatings containing TBT was stopped in Japan in 1997, it is still used in other countries and is a cause of pollution in seawater and marine life. These compounds are also suspected of being endocrine disruptors. The analysis of organotin compounds that have undergone standard alkylation and deuterium-labeled organotin compound mixtures is described here as an example.

■Analytical Conditions

Instrument:	: GC-17AAFwver.3 (FPD-17c Sn Filter)
Column	: DB-5 (30m × 0.25mm I.D., df = 0.25µm)
Column Temp.	: 60˚C(1min)-20˚C/min-140˚C -7˚C/min-280˚C(5min)
Inj. Temp.	: 290˚C
Det. Temp.	: 300˚C
Carrier Gas	: He, 35kPa(1min)-150kPa(2.4mL/min)
Injection method	: High-pressure splitless (1min)
Injection volume	: 3µL

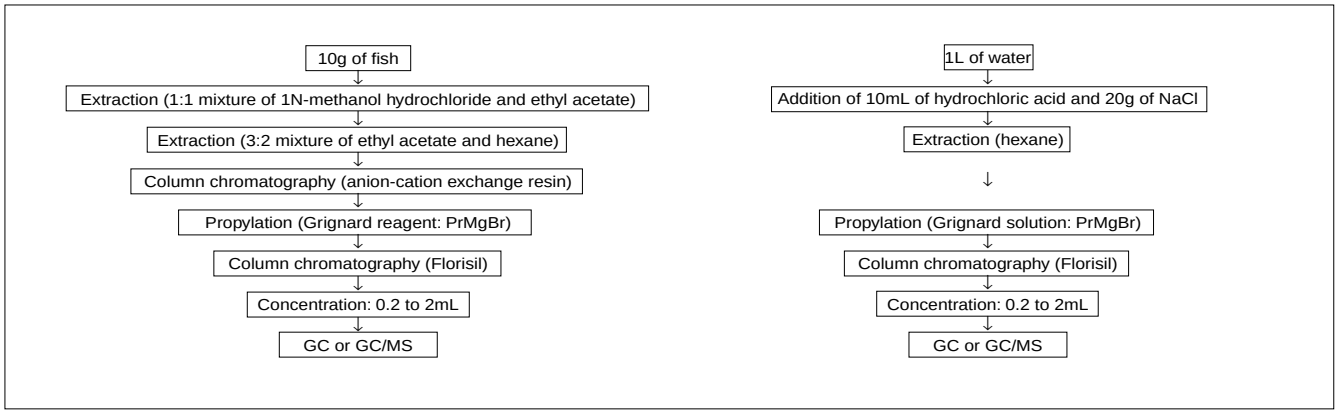


Fig. 4.6.1 Examples of extraction methods for organotin compounds

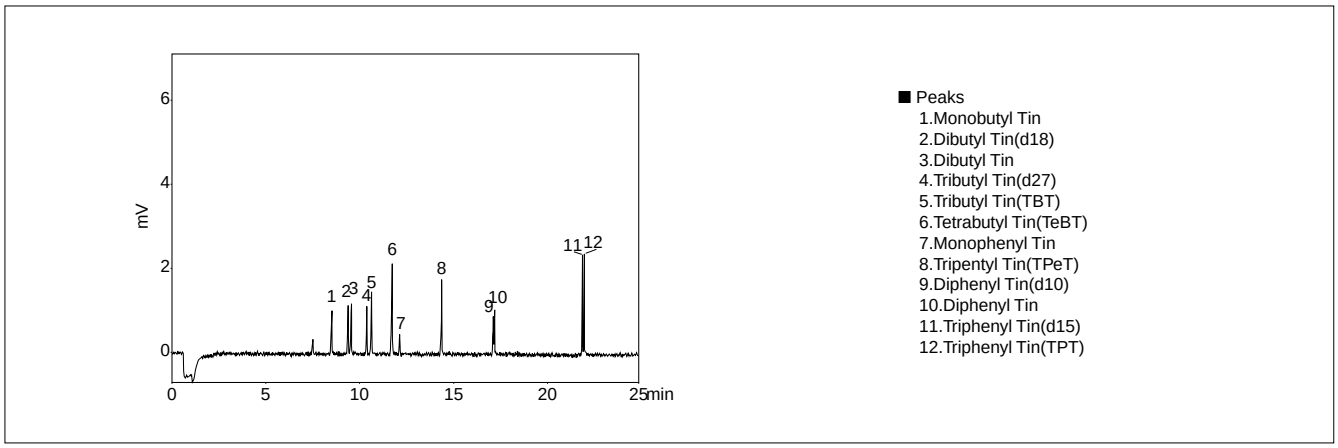


Fig. 4.6.2 Chromatogram of sea bass extract (0.2 to 0.4µg of standards added to 2mL of final solution)

4.7 Analysis of Carbon Tetrachloride Contained in Margarine - GC

■Analytical Conditions

Column	: CP-Sil 8 CB (Chrompack) 50m × 0.32mmI.D. df = 1.2µm
Carrier Gas	: He 120kPa
Column temperature	: 35°C (10min) -15°C/min -150°C
Detection	: ECD
Purge & Trap	: Tenax TA 150 mg commercial margarine

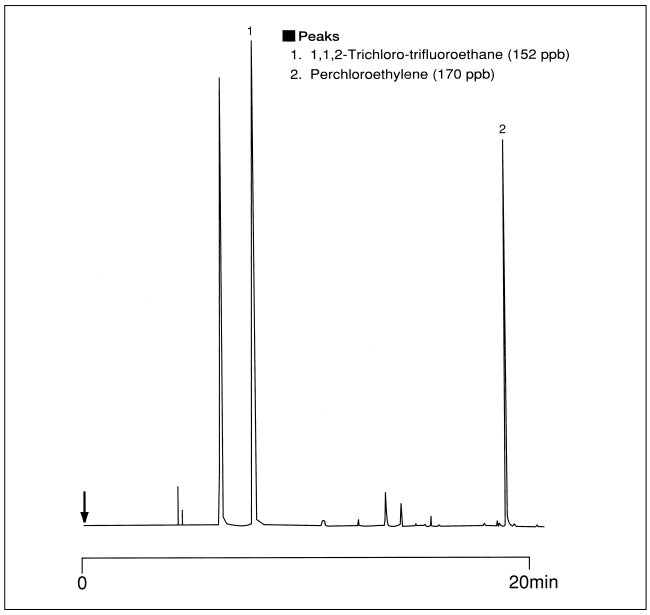


Fig. 4.7.1 Analysis of Carbon Tetrachloride Contained in Margarine
(Reprinted from J. of Chromatographic Science, Vol. 31, Dec. 1993)

4.8 Analysis of Nitrosamines - GC

Analytical Conditions

Column	: Rtx-200 (Restek) 30m × 0.53mmI.D. df = 0.5µm
Carrier Gas	: H_2 40cm/sec
Column temperature	: 100°C (1min) -5°C/min -200°C
Injection method	: Split (1: 40)
Detection	: FID
Injection volume	: 1 µL mixture containing 10 µg of nitrosamines

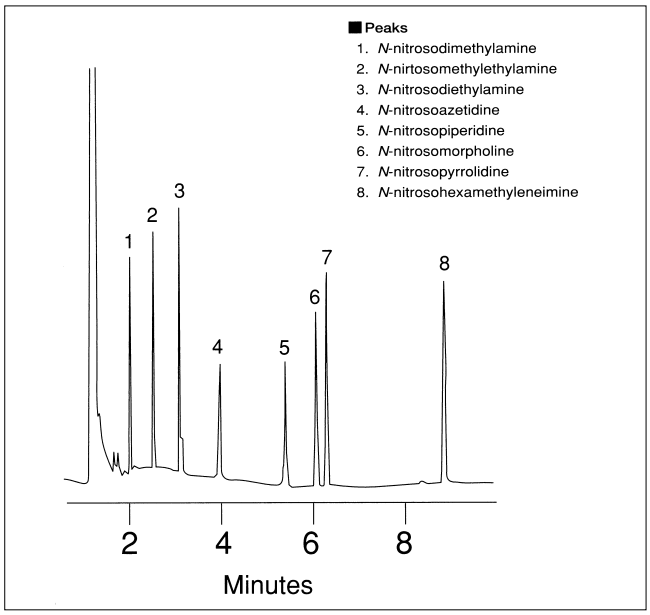


Fig. 4.8.1 Nitrosamines
(Reprinted from J. of Chromatographic Science, Vol. 31, Dec. 1993)

4.9 Analysis of Alkyl Mercury Compounds (1) - GC

■Explanation

There are two methods for measuring mercury in the environment - one is the method of measuring total mercury using UV and atomic absorption, and the other measuring organic mercury using GC. The GC method is especially useful for analyzing organic mercury, which was the cause of the Minamata disease in Japan. Most organic mercury in the environment exists as methyl mercury or ethyl mercury, which can be analyzed with high sensitivity and selectivity using ECD after chlorination.

References

Environmental Water Quality Analysis Method Manual 411 - 416 edited by the Environmental Chemical Research Association, Japan

■Analytical Conditions

Column	: Thermon-HG 10% on Chromosorb W (AW-DMCS) 80-100 mesh 0.5m × 3.0 mm I.D.
Column temperature	: 150°C
Injection inlet temperature	: 250°C
Detector temperature	: 280°C (ECD)
Carrier Gas	: N_2 40 mL/min

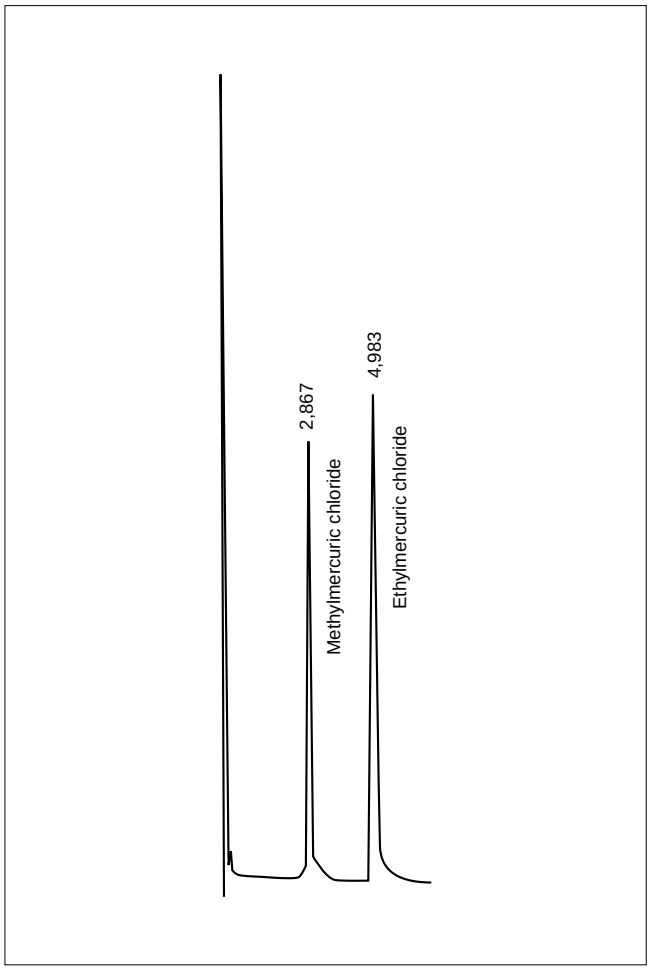


Fig. 4.9.1 Standard Sample (100 ppm each)

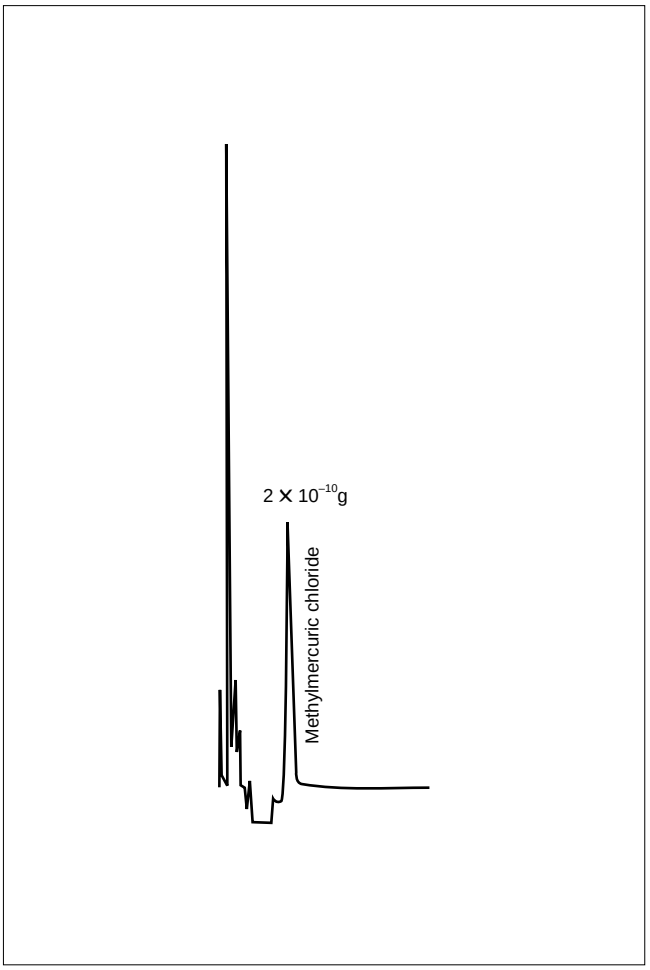


Fig. 4.9.2 Methyl Mercury contained in Snakehead Mullet caught in river A

4.9 Analysis of Alkyl Mercury Compounds (2) - GC

200 mL sample (use a 500 mL separatory funnel)

If not neutral, use ammonia water or hydrochloric acid to neutralize. Then add hydrochloric acid to provide approximately 2N.

Extraction

Add 50 mL of benzene, shake vigorously for 2 minutes, transfer the water layer to a different separatory funnel and save the benzene layer. Once again add 50 mL of benzene to the water layer, shake vigorously for 2 minutes and discard the water layer.

Washing the benzene layer

Combine the benzene layers, add 20 mL of sodium chloride solution (200 g/L), wash the benzene layer by stirring for approximately 1 minute and discard the water layer.

Reversed Extraction

Add 8 mL of L-cystine and sodium acetate solution [1] to the benzene layer, shake vigorously for approximately 2 minutes, allow to stand, then transfer the water layer to a separatory funnel (20 - 30 mL).

Extraction

Add 2 mL of hydrochloric acid and 5 mL of benzene, shake vigorously for 2 minutes, allow to stand, then transfer the benzene layer to a test tube that has a joint valve.

GC Analysis

(1) L-cystine and sodium acetate solution:
Dissolve 1 g of L-cysteine hydrochloride monohydrate 0.8 g of sodium acetate trihydrate and 12.8 g of sodium sulfate (anhydrous) into water to make 100 mL of solution.

Fig. 4.8.3 Flowchart for Alkyl Mercury Pretreatment

4.10 Analysis of Organotin in Fish (1) - GC/MS

■Explanation

Organotins such as tributyltin (TBT) and triphenyltin (TPT) are widely used as antifouling coatings for ships and fishing nets, and the resulting pollution of seawater and marine life has become an issue of concern. Although these compounds are usually analyzed using GC-FPD, analysis using GC/MS, which enables highly accurate qualitative determination, is described here as an example.

Although tripentyltin (TPeT) is often used with GC measurement as the internal standard substance, this is not an ideal selection because TBT, TPT, and TPeT have different recovery rates. In this example, a deuterium-labeled compound, which makes full use of GC/MS characteristics, is used as the internal standard substance. The advantage of the deuterium-labeled compound as a standard substance is that its properties are similar to the target compound but it does not exist in the sample.

■Pretreatment

The extraction methods for fish and seawater are shown below.

■Analytical Conditions

Instrument : GCMS-QP5000
Column : DB-1 (30m × 0.32mm I.D., df = 0.25μm)
Column Temp. : 50˚C(2min)-20˚C/min-140˚C-7˚C/min -220˚C-15˚C/min-310˚C(6min)
Inj. Temp. : 280˚C
I/F Temp. : 300˚C
Carrier Gas : He (40kPa)
Injection method : Splitless (2min)

Constituent	Selected ions (m/z)
d27-TBT	295, 293, 316
TBT	277, 275, 291
Tetra-BT	291, 289
TPeT	303, 305
d15-TPT	366, 364
TPT	351, 349

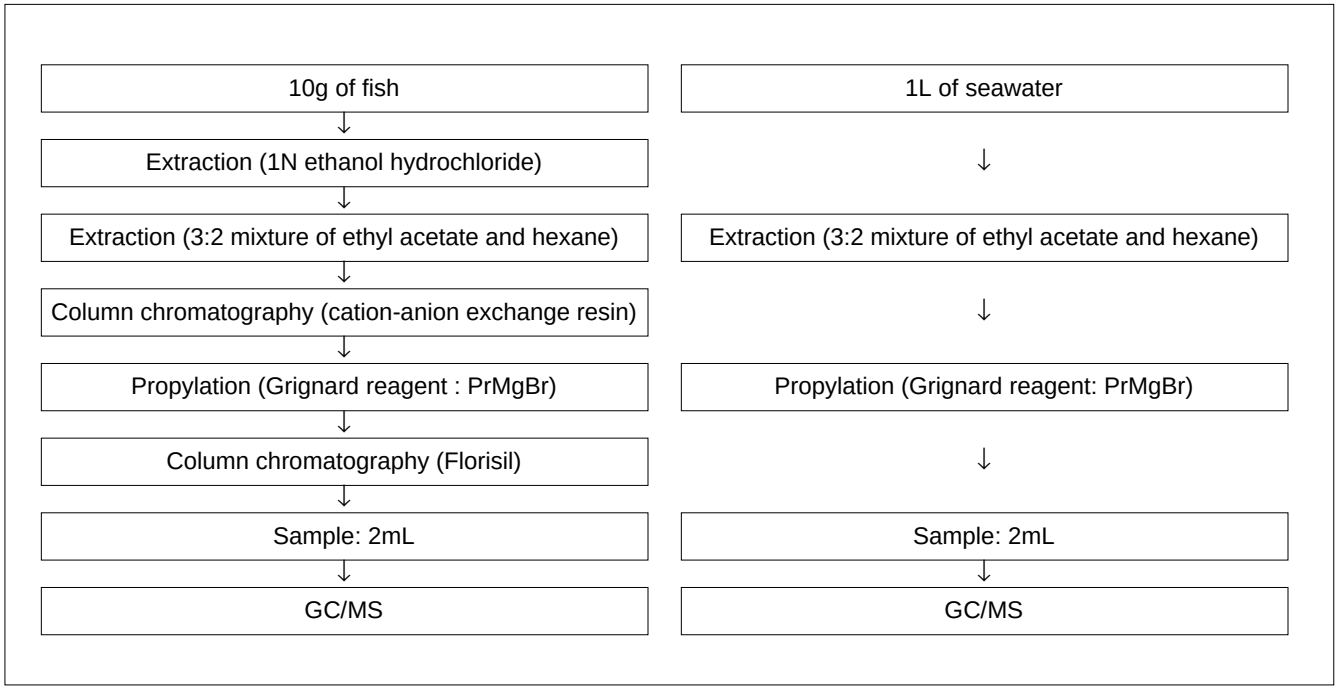


Fig. 4.10.1 Extraction methods for organotin in fish and seawater

4.10 Analysis of Organotin in Fish (2) - GC/MS

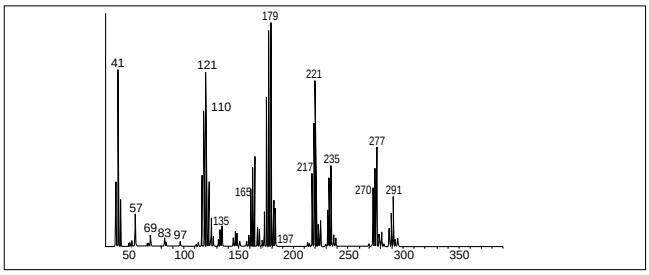


Fig. 4.10.2 Mass spectrum of TBT

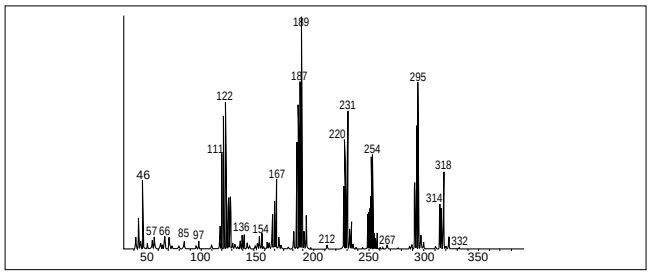


Fig. 4.10.3 Mass spectrum of d27-TBT

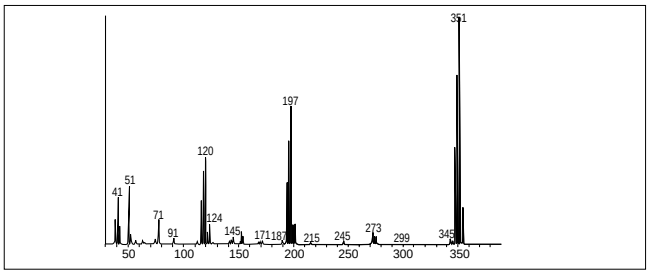


Fig. 4.10.4 Mass spectrum of TPT

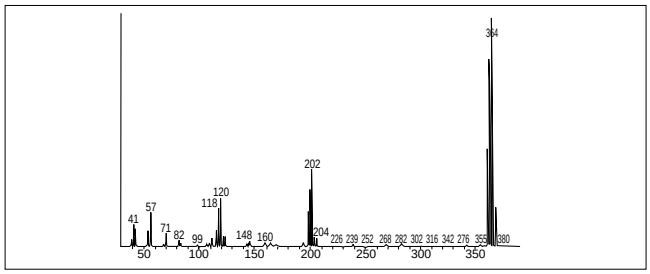


Fig. 4.10.5 Mass spectrum of d15-TPT

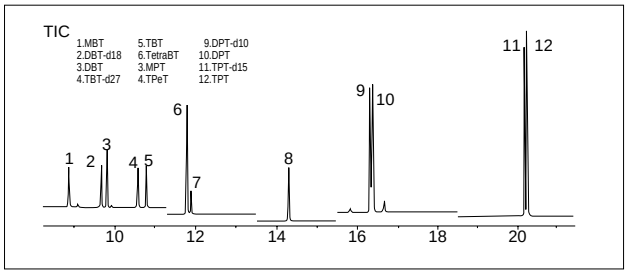


Fig. 4.10.6 SIM chromatogram of standard sample

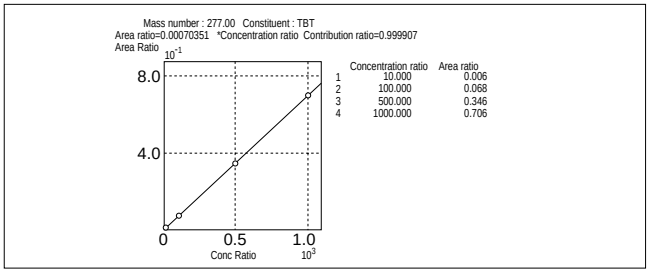


Fig. 4.10.7 Calibration curve for TBT (10 to 1,000ppb)

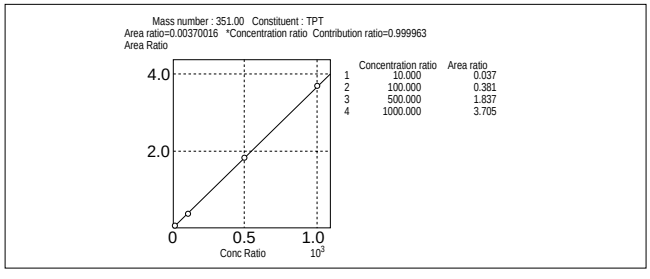


Fig. 4.10.8 Calibration curve for TPT (10 to 1,000ppb)

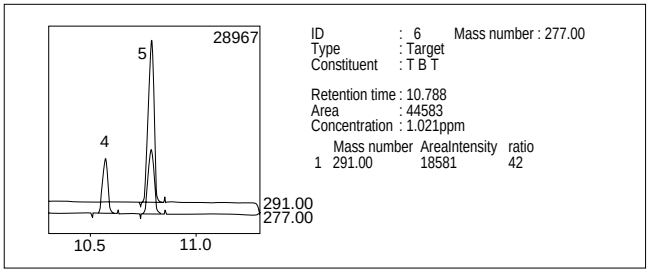


Fig. 4.10.9 SIM chromatogram for TBT in sea bass

Table 4.10.1 Quantitative Results for Tin in Fish and Seawater

Constituent	Sea bream (μg/g)	Sea bass (μg/g)	Port K (μg/L)	Port W (μg/L)
TBT	0.436	0.782	0.173	0.068
TPT	0.014	0.010	0.019	0.078

4.11 Analysis of 3-MCPD in Soy Sauce (1) - GC/MS

■Explanation

When the vegetable protein contained in barley, soybeans, and peanuts is subjected to high-temperature processing in the presence of hydrochloric acid, it is converted into acid-HVP (hydrolyzed vegetable protein). HVP plays an important part in seasoning and flavoring. In the conversion process, however, residual lipid constituents are converted into 3-MCPD. Although 3-MCPD has not been recognized as toxic to humans, it has been recognized as carcinogenic to animals, and it is desirable that the intake of this constituent is kept as small as possible.

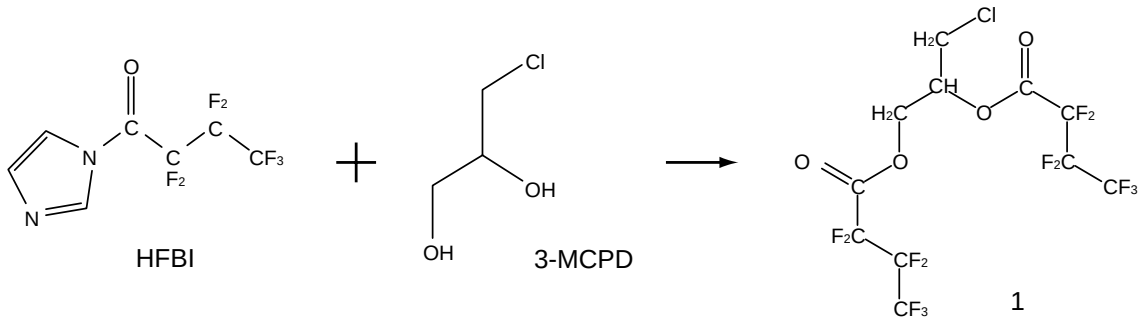


Fig. 4.11.1 Derivatization of 3-MCPD

■Pretreatment

Soy sauce 8g
↓ Total mass increased to 20g with NaCl.
Homogenized with homogenizer.
↓
Injected in Extrelut NT20 column.
↓ 75mL Hexane:Diethyl ether (90:10)
(rinsing of nonpolar substances)
↓ 250mL Diethyl ether,30min
(extraction of 3-MCPD)
Concentrated to 10mL (evaporator)
↓
Concentrated to 2mL (N_2 purge)
↓ +1mL Isooctane
↓ +50µL HFBI
70°C 20min Left at room temperature.
↓ +1mL Water
Vortex 30s, twice
↓
Upper organic layer transferred to vial.
↓ +Anhydrous sodium sulfate added.
GC/MS

■Analytical conditions

Instrument	: GCMS-QP5050A
Column	: DB-5, 30m × 0.25mm I.D., df = 0.25µm
Column temperature	: 50ºC (1min) - 2ºC/min - 90ºC - 40ºC/min - 270ºC
Carrier gas	: He, 100kPa
Injection-inlet temperature	: 270ºC
Injection method	: Splitless (sampling time: 0.6min)
Injection volume	: 1µL

<EI>

Interface temperature	: 270ºC
Measurement mode	: SIM
m/z	: 253, 275, 289, 291, 453

<NCI>

Reaction gas	: Isobutane
Reaction gas pressure	: 0.5bar
Interface temperature	: 200ºC
Measurement mode	: Scan/SIM
m/z (scan)	: 151-514 amu
m/z (SIM)	: 446, 482, 502 (3-MCPD)

4.11 Analysis of 3-MCPD in Soy Sauce (2) - GC/MS

■EI Mass Spectrum, Scan

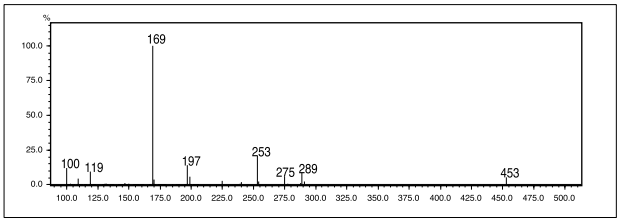


Fig. 4.11.2 EI Mass Spectrum
Full scan EI mass spectrum of HFBI derivative of 3-MCPD.

■NCI Mass Spectrum, Scan

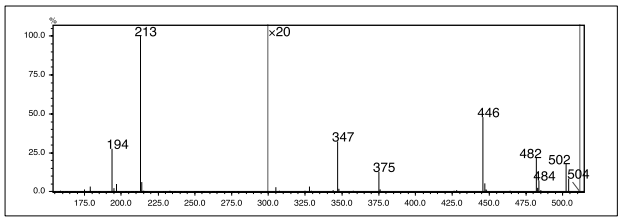


Fig 4.11.3 NCI Mass Spectrum
NCI isobutane mass spectrum of 3-MCPD derivative,
M = [$CH_2(COOCF_2CF_2CF_3)$ $CH(COOCF_2CF_2CF_3)CH_2Cl$].
The region from *m/z* 220-514 is magnified 20x.

■Monitoring Mass

Table 4.11.1 Monitoring Mass
Ions used for detection and quantitation of 3-MCPD
(Parent compound: M = [$CH_2(COOCF_2CF_2CF_3)$
$CH(COOCF_2CF_2CF_3)CH_2Cl$], M.W. = 502) and the possible chemical formula for the ion species.

Ion Species	*m/z*
$[M\text{-}CH_2\text{-}^{35}Cl]^+$	453
$[M\text{-}(COOCF_2CF_2CF_3)]^+$ ($Cl = {}^{37}Cl$)	291
$[M\text{-}(COOCF_2CF_2CF_3)]^+$ ($Cl = {}^{35}Cl$)	289
$[M\text{-}(COOCF_2CF_2CF_3)\text{-}CH_2]^+$ ($Cl = {}^{35}Cl$)	275
$[M\text{-}(COOCF_2CF_2CF_3)\text{-}H^{35}Cl]^+$	253
$[CF_3CF_2CF_2]^+$	169

■Monitoring Mass

Table 4.11.2 Monitoring Mass
Ions observed in the NCI of 3-MCPD derivative
(M = [$CH_2(COOCF_2CF_2CF_3)$ $CH(COOCF_2CF_2CF_3)CH_2Cl$], M.W. = 502).

	3-MCPD	
Ion Species	*m/z*	Rel. Int.(%)
$[M]^-$ ($Cl = {}^{35}Cl$)	502	37
$[M]^-$ ($Cl = {}^{37}Cl$)	504	17
$[M\text{-}HF]^-$ ($Cl = {}^{35}Cl$)	482	45
$[M\text{-}HF]^-$ ($Cl = {}^{37}Cl$)	484	15
$[M\text{-}HF\text{-}HCl]^-$	446	100
$[CF_3CF_3CF_2COO]^-$	213	4181

■EI Mass Chromatogram, SIM

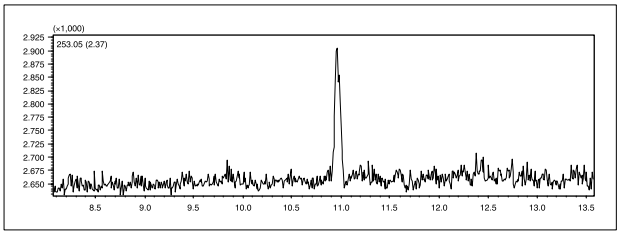


Fig. 4.11.4 m/z 253 Mass Chromatogram
Mass chromatogram of *m/z* 253 ion (3-MCPD = 5ng/mL) obtained by Electron impact Ionisation in SIM acquisition mode.

■NCI Mass Chromatogram, SIM

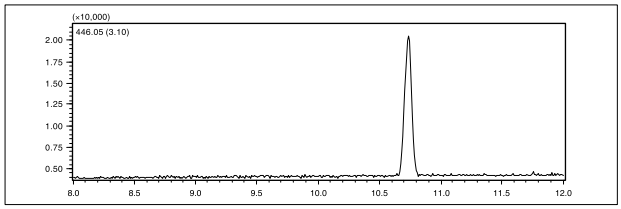


Fig. 4.11.5 m/z 446 Mass Chromatogram
Mass chromatogram of *m/z* 446 ion (3-MCPD = 5ng/mL) obtained by Negative Chemical Ionisation in SIM acquisition mode.

■Calibration Curve, EI

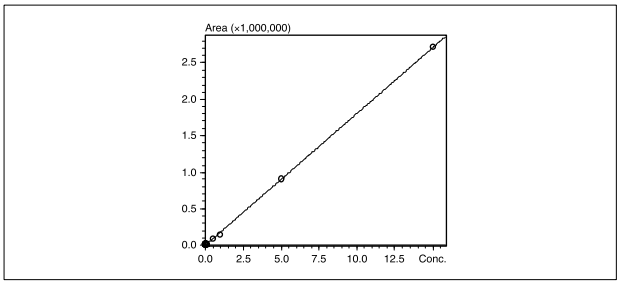


Fig. 4.11.6 Calibration Curve(5-15000ng/mL)EI
External standard calibration plot for 3-MCPD analysis by EI-Scan in the concentration range 5-15000ng/mL. using *m/z* 253 ion as the quantitation ion.
$y = 181066.2x$. $r^2 = 0.9997526$. $r = 0.9998763$.

■Calibration Curve, NCI

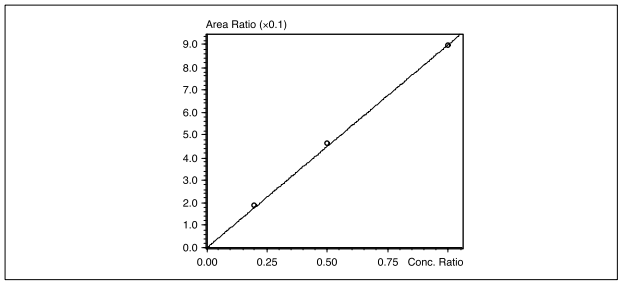


Fig. 4.11.7 Calibration Curve(2-20ng/mL)NCI
Internal standard calibration plot for 3-MCPD analysis by NCI-Scan in the concentration range of 2-10ng/mL: using *m/z* 213 ion as the quantitation ion.
$y = 0.9023328x$. $r^2 = 0.9999215$. $r = 0.9999607$.

4.12 Analysis of Pb in Milk Using Atomic Absorption Spectrophotometry - AA

■Explanation

Lead is harmful to human body and stricter regulations are being applied to lead in food and pharmaceutical products. Lead can be effectively detected by electrothermal atomization with atomic absorption.

Analysis of Pb in milk generally involves the flame method or electrothermal atomization where an acid is added and the sample is thermally decomposed. However, these methods require time-consuming pretreatment.

With direct analysis using electrothermal atomization, oxygen is often added during incineration to enhance the decomposition of organic matter in milk. However, the oxygen causes the deterioration of the graphite tube.

Here, the use of a platform tube, instead of the graphite tube, allowed accurate measurement without the addition of oxygen or air.

■Analytical Conditions

Wavelength	: Pb 283.3nm
Lamp current Low (mA)	: 10
Lamp current High (mA)	: 0
Slit width (nm)	: 0.5
Background correction	: BGC-D_2

Table 4.12.1 Heat program

Air not added

Stage	Temperature (°C)	Time (sec)	Heat mode	Gas	Inner gas flow rate
1	70	3	lamp	Ar	0.20
2	120	30	lamp	Ar	0.50
3	400	20	lamp	Ar	0.50
4	500	10	lamp	Ar	1.00
5	700	10	step	Ar	1.00
6	700	3	step	Ar	0.0H
7	2400	3	step	Ar	0.0H
8	2600	2	step	Ar	1.00

Table 4.12.2 Measurement results for Pb in milk

	Measurement results	Added amount
Air added	10.5ppb	10.0ppb
Air not added	10.4ppb	10.0ppb

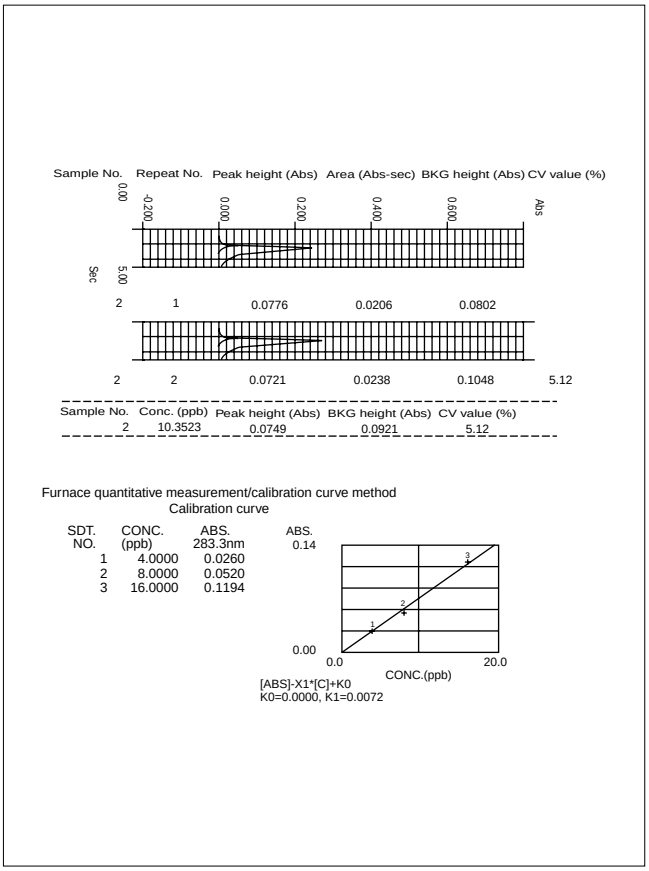


Fig. 4.12.1 Peak profile and calibration curve of Pb in milk

4.13 Analysis of Pb in White Sugar Using Atomic Absorption Spectrophotometry (1) - AA

■Explanation

Lead is harmful to human body and stricter regulations are being applied to lead in food and pharmaceutical products. Lead can be effectively detected by the electrothermal atomization with atomic absorption. The 13th revision of the Japanese Pharmacopoeia requires the measurement of lead, instead of heavy metal, using the electrothermal atomization method in purity tests for refined white sugar.

Here, analysis was performed in accordance with the Pharmacopoeia, with pretreatment (see Fig. 4.13.1) and sample preparation using an autosampler for the standard addition method.

Table 4.13.1 shows the measurement parameters and Fig. 4.13.2 shows the measurement results. Lead was not detected in the analyzed white sugar, but 1ppb of lead was clearly detected in the calibration curve. It can be said that this analysis method is effective for the detection of 0.5 ppm lead in white sugar (5ppb or less in processed solution), which is specified in the standard.

■Analytical Conditions

Wavelength : Pb 283.3nm
Lamp current Low (mA) : 10
Lamp current High (mA) : 0
Slit width (nm) : 0.5
Background correction : BGC-D_2

Accurately load 0.050g of sample into
polytetrafluoroethylene container
↓
Add 0.5mL of nitric acid to dissolve sample
↓
Seal container and heat for 5 hr at 150°C
↓
Cool, add water to accurately make 5mL of sample solution
↓
Analyze using standard addition method for AA
(electrothermal)

Fig. 4.13.1 Pretreatment for Pb in refined white sugar

Table 4.13.1 Measurement parameters

Lighting Conditions

Element :	Pb
Turret No. :	1
Lamp current Low (mA) :	10
Lamp current High (mA) :	0
Wavelength (nm) :	283.3
Slit width (nm) :	0.5
Lighting mode :	BGC-D2

Temperature Program

Final stage No. of concentration in oven : 5
Concentration frequency : 1

	Temperature (°C)	Time (sec)	Heat mode	Sensitivity	Gas	Inner gas flow rate	Sampling	Previous stage (sec)
1	110	30	Ramp	Regular	Gas #1	0.20	Off	0
2	250	10	Ramp	Regular	Gas #1	0.20	Off	0
3	600	20	Ramp	Regular	Gas #1	1.00	Off	0
4	600	20	Step	Regular	Gas #1	1.00	Off	0
5	600	5	Step	High	Gas #1	0.00	Off	0
6	2100	3	Step	High	Gas #1	0.00	On	2
7	2600	2	Step	Regular	Gas #1	1.00	Off	0

Autosampler Mixing Conditions

Adding Conc. (ppb)	Sample amount	R2 (Pb: 10ppb standard solution)	R1 (pure water)	Total
Blank	0µL	0µL	200µL	200µL
0	100µL	0µL	100µL	200µL
1	100µL	20µL	80µL	200µL
2	100µL	40µL	60µL	200µL
3	100µL	60µL	40µL	200µL

Pb: 10ppb standard solution and pure water containing approximately 1.1mol/L of nitric acid
Inj. Vol. : 20µL

4.13 Analysis of Pb in White Sugar Using Atomic Absorption Spectrophotometry (2) - AA

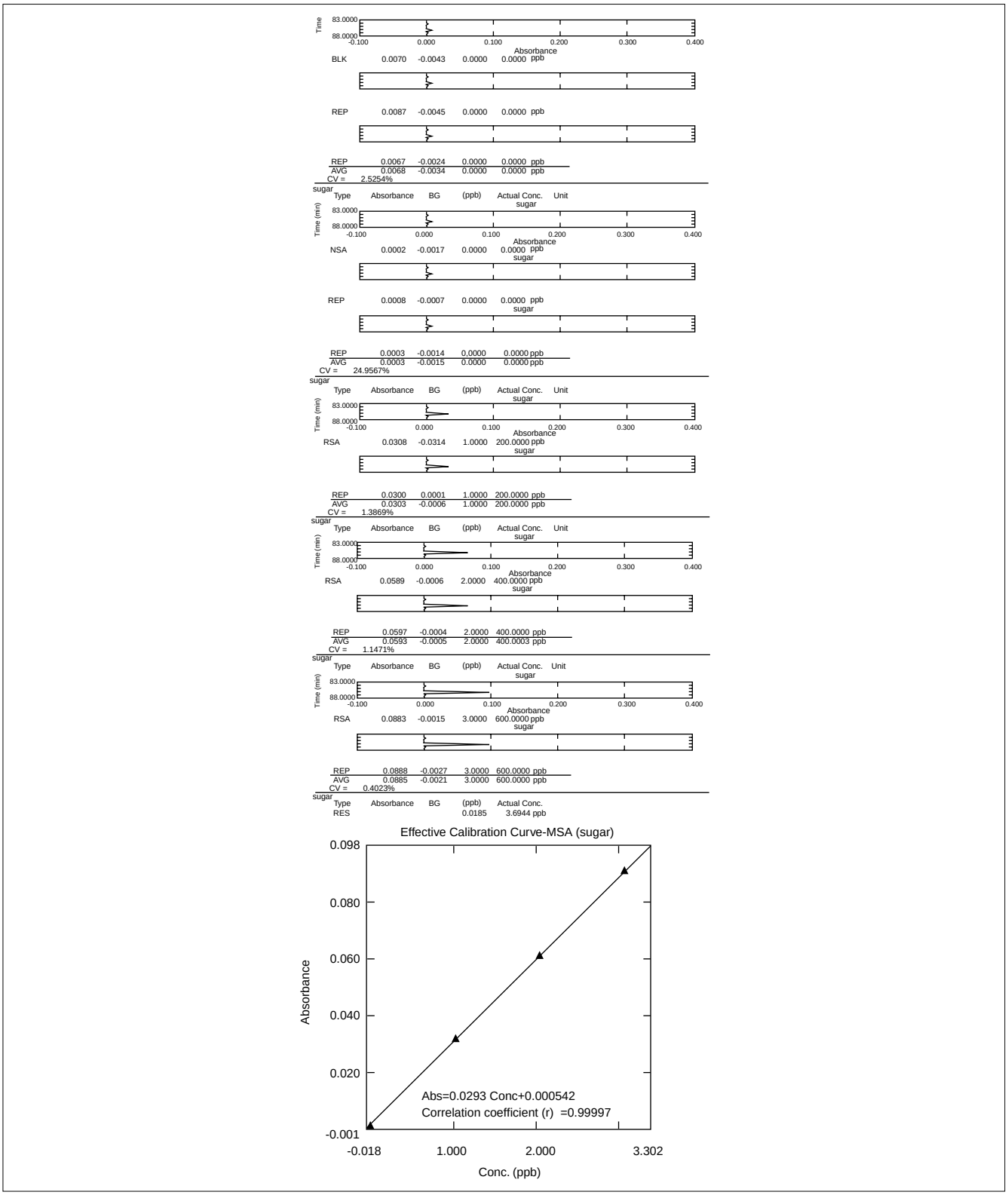


Fig. 4.13.2 Measurement results for Pb in refined white sugar

4.14 Measurement of Metals Contained in Food Additives (1) - AA

■Explanation

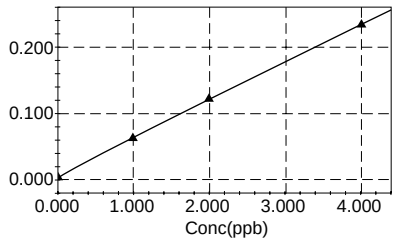
Japanese regulations about food additives were revised on April 6, 1999. In conjunction with this revision, new test methods were stipulated for newly regulated items and the former tests methods were revised for some items.

The method for analyzing arsenic in tar pigments was also revised. In this revision, the use of the hydride generator with sodium tetrahydroborate as a reducing agent for atomic absorption spectrometry was newly added.

This section shows an example of analyzing the red tar pigment No. 104. Arsenic was analyzed using the hydride generation method, while zinc, iron and lead were analyzed using the flame method. As for lead, the result obtained by using the optional booster is also shown.

Sample pretreatment was performed in accordance with the official method. 2.5 grams of the sample was placed in a platinum crucible, sulfuric acid was added and the mixture was incinerated at a low temperature on a hot plate. Then, after incineration at 550°C in an electric oven, the ash was dissolved in hydrochloric acid to produce the sample solution.

Calibration curve (calibration curve #: 01)



Conc (ppb)	Abs
0.0000	0.0023
1.0000	0.0648
2.0000	0.1251
4.0000	0.2386

Abs=-0.00116Conc ^2+0.0637Conc+0.0023
r=1.0000

STD 1 : STD

Concentration setting	Absorbance	BG
0.0000	0.0023	0.0026

STD 2 : STD

Concentration setting	Absorbance	BG
1.0000	0.0648	0.0049

STD 3 : STD

Concentration setting	Absorbance	BG
2.0000	0.1251	0.0072

STD 4 : STD

Concentration setting	Absorbance	BG
4.0000	0.2386	0.0086

Red pigment No. 104: UNK

Concentration	Absorbance	BG	Yield Qty	Supplement Qty	Actual Conc.
0.0285	0.0041	0.0098	0.500000	25.00	1.4250

Fig. 4.14.1 Measurement of Arsenic

4.14 Measurement of Metals Contained in Food Additives (2) - AA

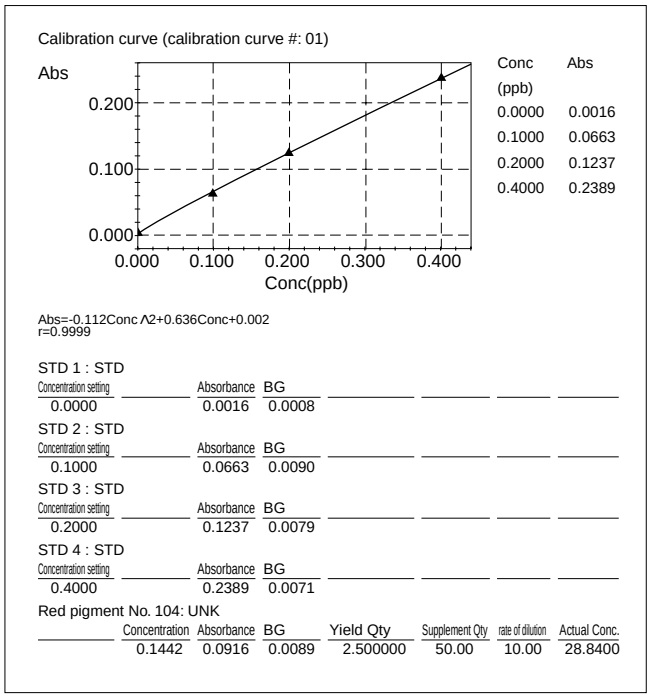


Fig. 4.14.2 Measurement of Zinc

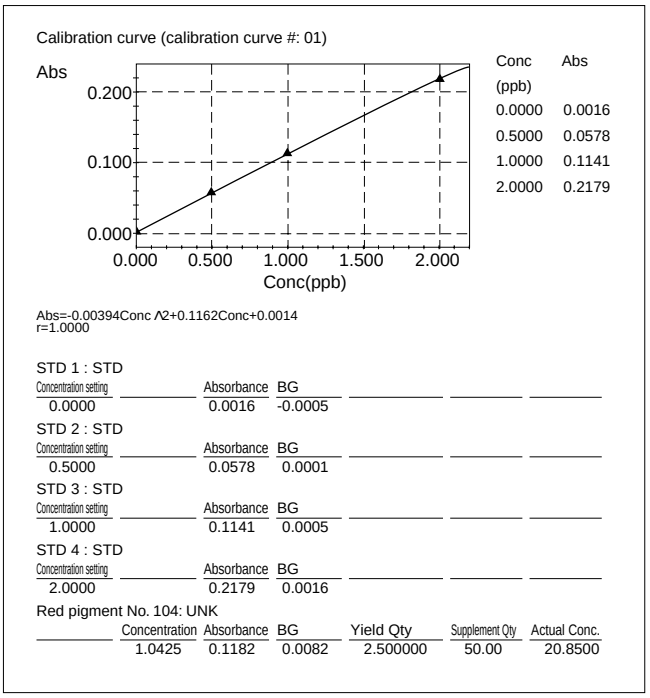


Fig. 4.14.3 Measurement of Iron

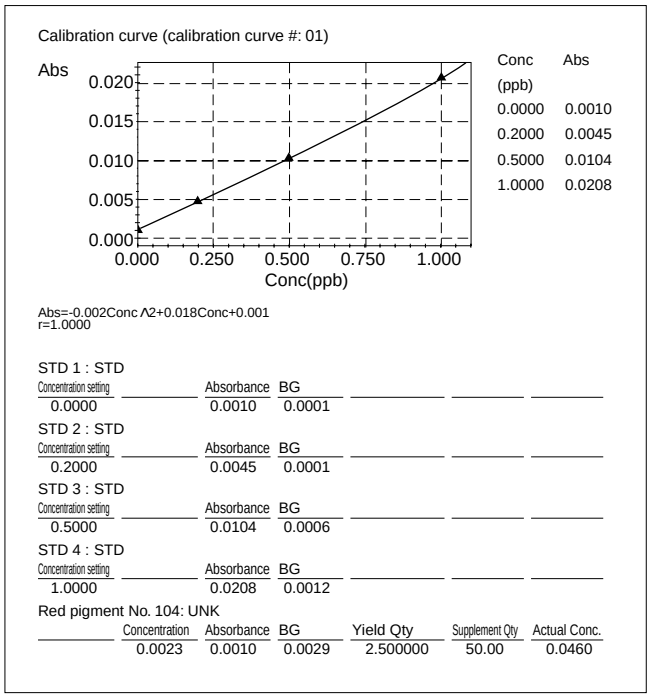


Fig. 4.14.4 Measurement of Lead

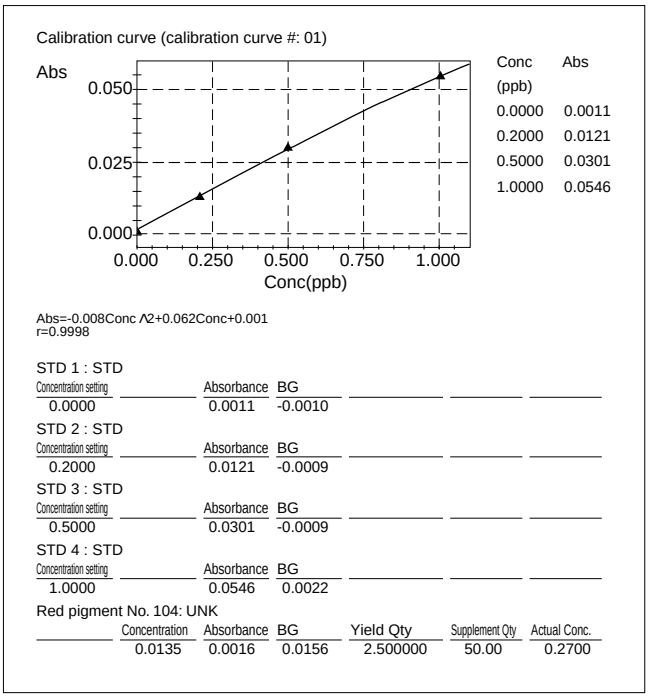


Fig. 4.14.5 Measurement of Lead (with booster)

The sensitivity of the flame analysis of lead increases by about three times by using the booster. This revision also employs electric heating (flameless analysis) as an alternate method for analyzing lead. It indicates methods such as (1) adding standards and (2) adding palladium nitrate to suppress interference.

4.15 Analysis of Inorganic Components in Canned Drink (Green Tea) (1) - ICP-AES

■Explanation

Samples like green tea can be directly inducted for ICP and AA analysis without pretreatment as long as there is no sediment. Here, inorganic components in shop-sold canned drink (green tea) were qualitatively and quantitatively analyzed using an ultrasonic nebulizer with the ICP-AES.
Here, semi-quantitative values and spectrum line profiles were obtained for approximately 72 elements with qualitative analysis. Almost identical quantitative results were obtained for the directly inducted sample and the sample treated by conventional wet decomposition.

References

- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

■Pretreatment

1. Direct introduction sample
   After opening seal, place 50mL of sample in plastic container, add 1mL of nitric acid and an internal standard element Y to 100ppb, and agitate the mixture.
2. Wet decomposition sample
   After opening seal, place 50mL of sample in a 100mL beaker and boil on a hotplate (approximately 190°C). When the whole sample has been reduced to 10mL, add 5mL of nitric acid and 1mL of hydrochloric acid and thermally decompose it for approximately 2 hours. After cooling, add ultra pure water to make it exactly 50mL and agitate it.

■Analytical Conditions

Instrument	: ICPS-8000
	: Ultrasonic nebulizer UAG-1
High frequency	: 27.12MHz
High frequency output	: 0.8kW
Cooling Gas	: Ar 14.0L/min
Plasma Gas	: Ar 1.2L/min
Carrier Gas	: Ar 0.7L/min
Purge Gas	: Ar 3.5L/min
Sample suction rate	: 1.5mL/min
Observation method	: Horizontal

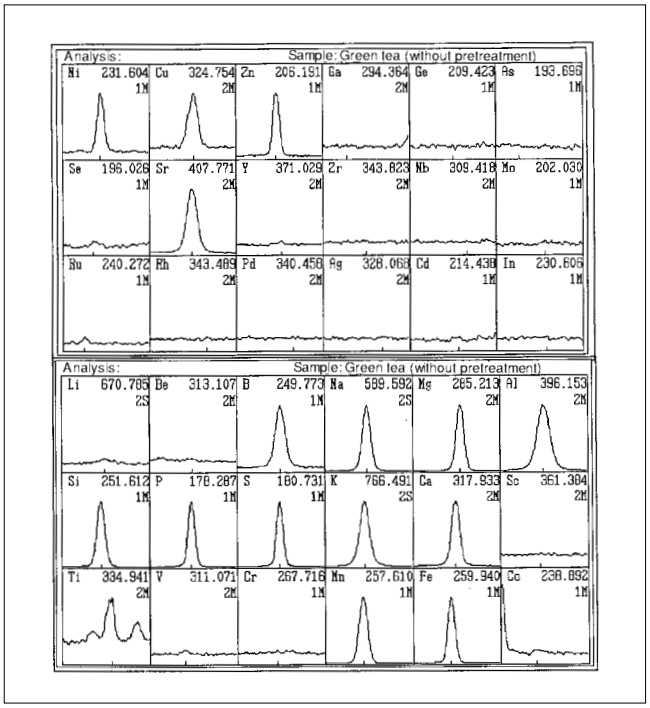


Fig. 4.15.1 Profile example for qualitative analysis

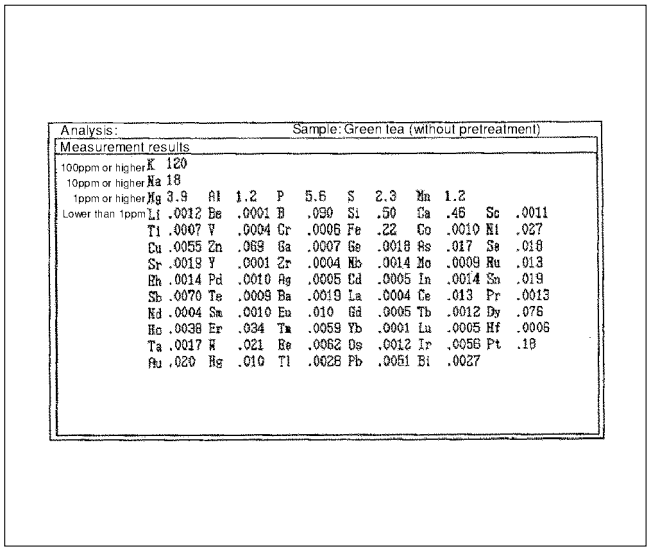


Fig. 4.15.2 Semi-quantitative value for qualitative analysis

4.15 Analysis of Inorganic Components in Canned Drink (Green Tea) (2) - ICP-AES

Table 4.15.1 Green tea analysis results (µg/mL)

Element	Direct introduction	Wet decomposition
Fe	0.249	0.260
Ni	0.029	0.029
Al	1.27	1.30
Pb	<0.001	<0.001
Sn	<0.001	0.002
Cu	0.0057	0.0053
Zn	0.083	0.089
Cr	0.0008	0.0007

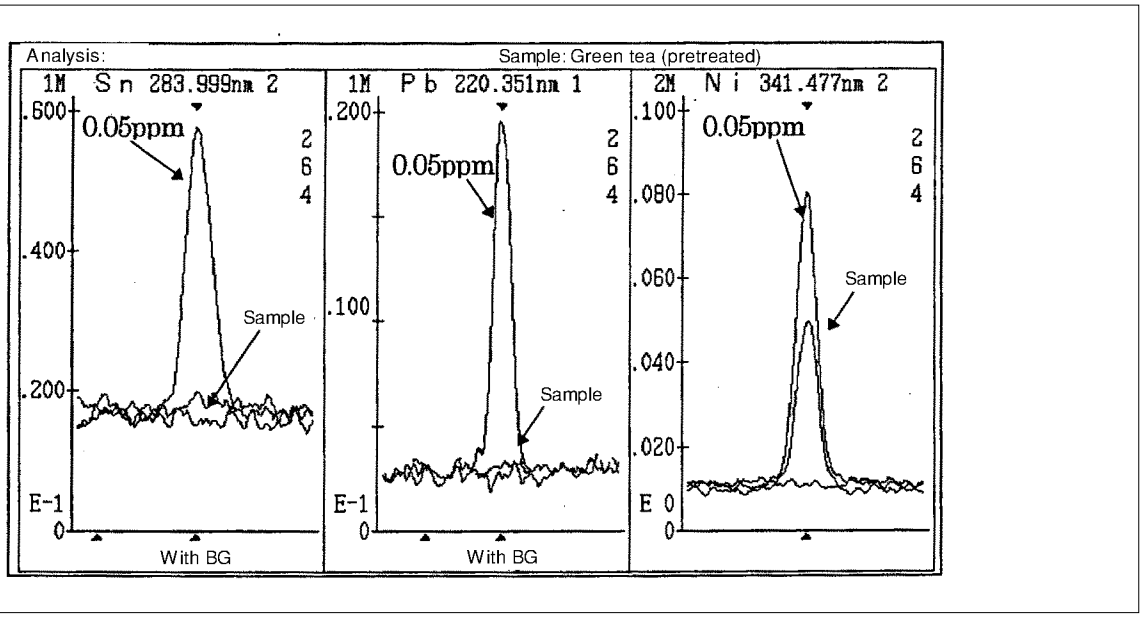


Fig. 4.15.3 Spectrum line profile

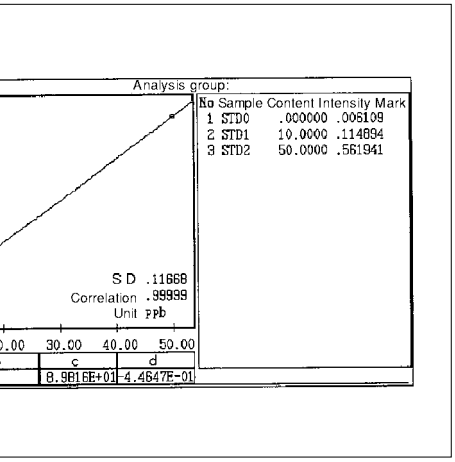


Fig. 4.15.4 Pb calibration curve

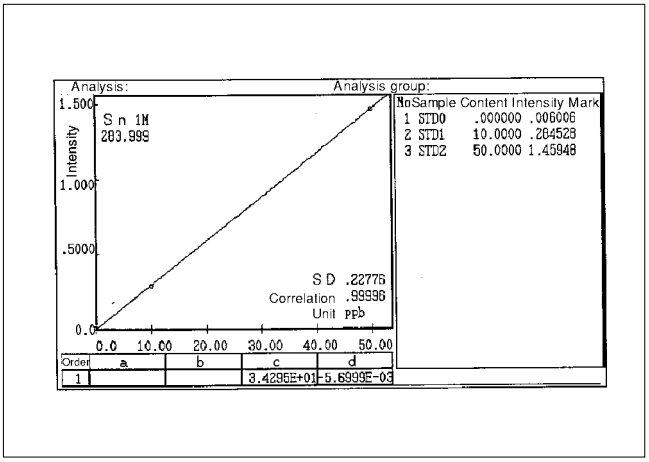


Fig. 4.15.5 Sn calibration curve

4.16 Analysis of Inorganic Components in Processed Food Products - ICP-AES

■Explanation

This is an analysis example for processed food. Various elements included in food products are divided into essential ones and harmful ones. The ICP emission spectrometry, which allows simultaneous analysis of these elements, is quite useful for comprehending the mutual relationships between elements.

Samples

Tuna, bean curd dressed with liquid starch, vegetables boiled in miso, rice and vegetable porridge, rice gruel

References

- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

■Pretreatment

Homogenize each sample in a homogenizer, take 10g for each, add 10mL of nitric acid and 2mL of sulfuric acid and thermally decompose them until white smoke of sulfuric acid appears. After cooling, measure up to 100mL. Use these as samples.

■Analytical Conditions

Instrument	: ICPS-8000
High frequency	: 27.12MHz
High frequency output	: 1.2kW
Cooling Gas	: Ar 14.0L/min
Plasma Gas	: Ar 1.2L/mln
Carrier Gas	: Ar 0.7L/min
Purge Gas	: Ar 3.5L/min
Sample suction rate	: 0.6mL/min
Observation method	: Horizontal
Sample induction	: Coaxial nebulizer/ double tube chamber

Table 4.16.1 Analysis results (μg/g: wet weight)

Element	Tuna	Bean curd dressed with liquid starch	Vegetables boiled in miso	Rice and vegetable porridge	Rice gruel
Na	560	1150	1610	972	10.3
Mg	77	874	103	18.9	10.9
P	460	303	351	83	47.8
K	747	603	628	82	38.9
Ca	23.3	145	219	22.3	10.5
Mn	0.13	1.01	1.20	0.48	0.43
Fe	2.10	3.25	3.40	0.36	0.21
Zn	1.16	2.39	2.35	1.21	1.21
Cd	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Pb	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

4.17 Analysis of Inorganic Components in Powdered Milk (1) - ICP-AES

■Explanation

The microwave sample decomposition method is quicker than the conventional wet decomposition method and takes place in a sealed system to prevent external contamination and volatilization loss of components such as As and Se. It is an extremely useful method to decompose the sample when the sample amount is small, or when a micro-amount element is to be analyzed.

Here, powdered milk was liquidized using a microwave decomposition unit and analyzed using ICP-AES. The ICP-AES, which causes little self-absorption and has a wide dynamic range, enables analysis of major components like sodium and calcium, as well as minor components such as cadmium and lead, in the same solution. Arsenic, selenium and antimony can be analyzed at higher sensitivity by using a hydride generator.

■Analytical Conditions

Instrument	: ICPS-7500
	: HVG-1 (hydride generator)
High frequency	: 27.12MHz
High frequency output	: 1.2kW
Cooling Gas	: Ar 14.0L/min
Plasma Gas	: Ar 1.2L/min
Carrier Gas	: Ar 0.7L/min
Purge Gas	: Ar 3.5L/min
Sample suction rate	: 0.6mL/min (Hydride generating method: 2.5mL/min)
Observation method	: Horizontal/axial
Sample injection system	: Coaxial nebulizer/cyclone chamber, hydride generator

■Pretreatment

See Fig. 4.17.1 for details of the operation flow for microwave decomposition.

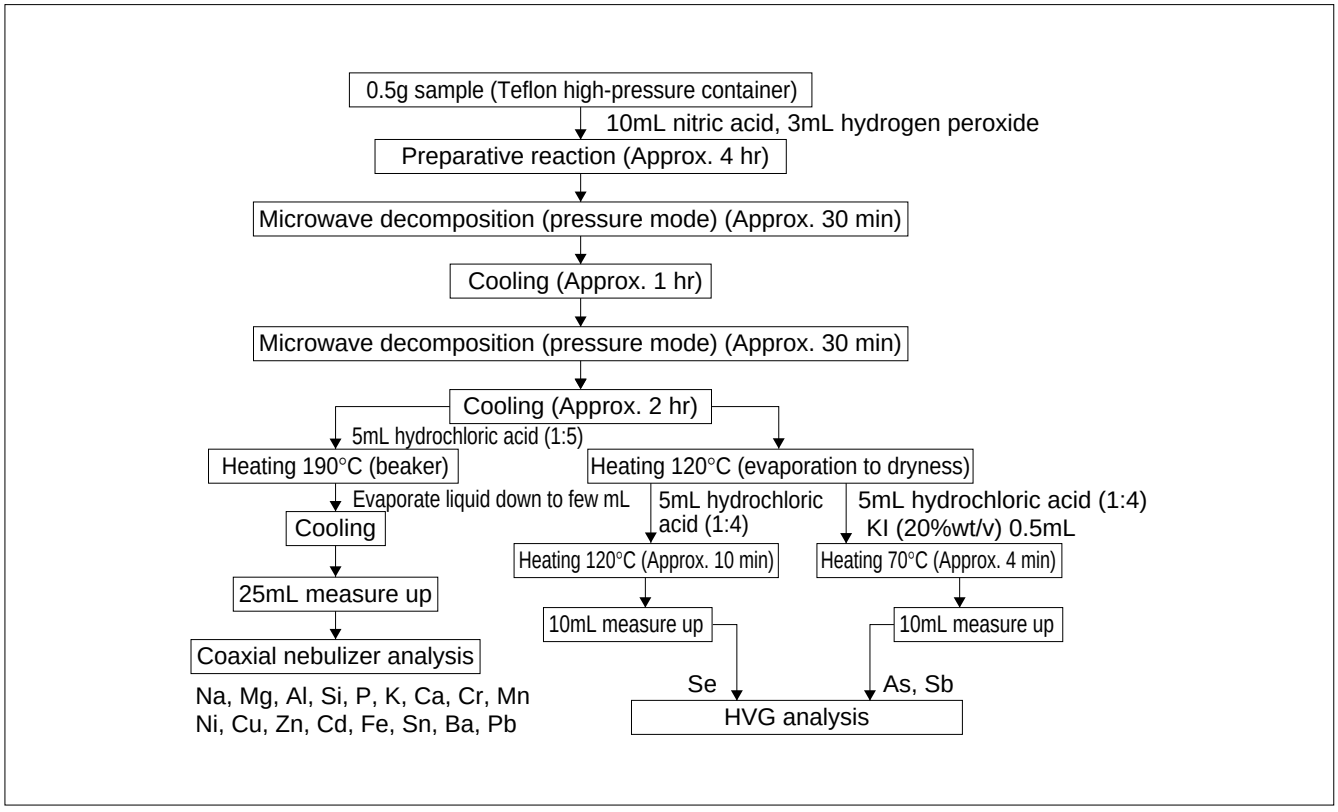


Fig. 4.17.1 Microwave decomposition flowchart

4.17 Analysis of Inorganic Components in Powdered Milk (2) - ICP-AES

Table 4.17.1 Powdered milk analysis results (µg/g)

Element	Measured value	Element	Measured value
Na	1259	Si	23
Mg	376	Ba	1.4
P	2238	Ni	0.21
K	4644	Sn	0.2
Ca	3960	Cr	0.04
Mn	0.34	Cd	0.022
Fe	75	Pb	<0.5
Cu	2.8	As	0.007*
Zn	23	Sb	0.002*
Al	3.0	Se	0.03 *

* HVG hydride generator used

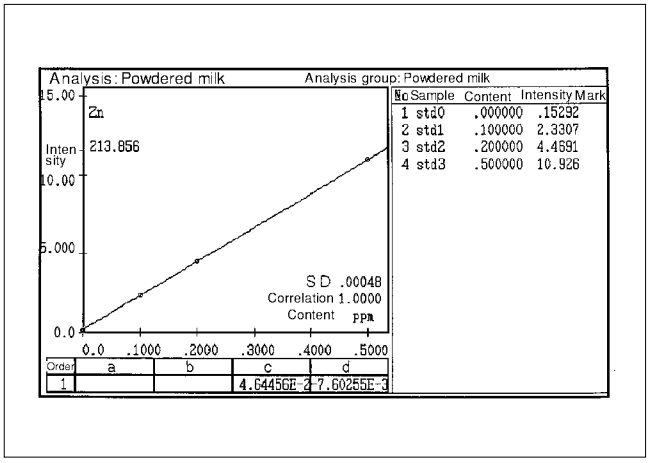


Fig. 4.17.2 Zn calibration curve

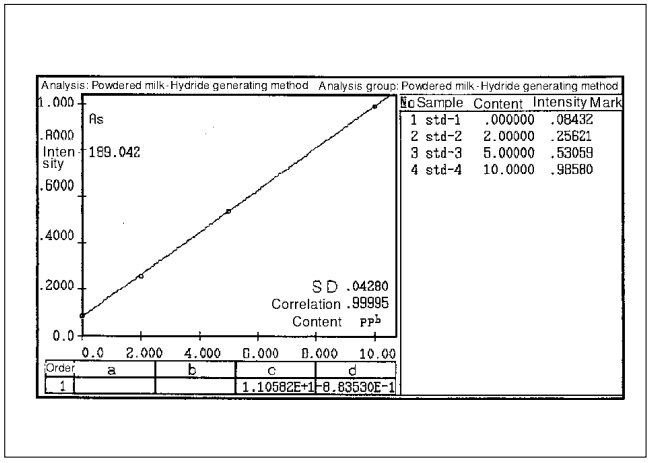


Fig. 4.17.3 As calibration curve

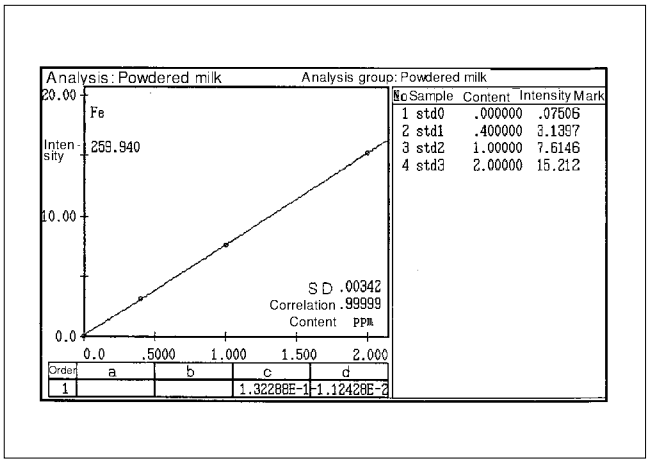


Fig. 4.17.4 Fe calibration curve

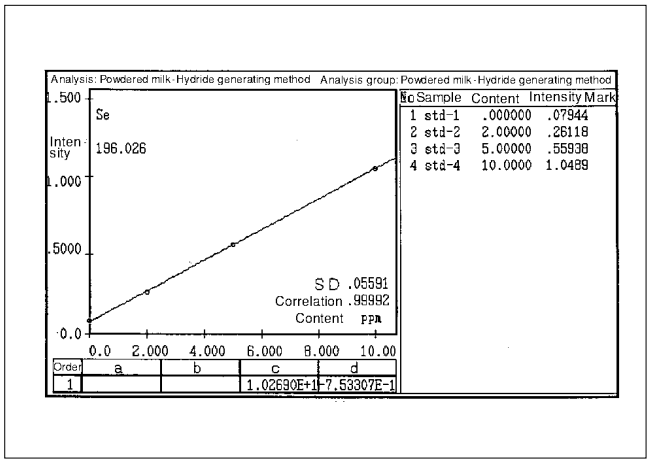


Fig. 4.17.5 Se calibration curve

4.17 Analysis of Inorganic Components in Powdered Milk (3) - ICP-AES

■Explanation

Here, a standard powdered milk was analyzed after incineration. The results show that nearly all the inorganic components conformed to the guaranteed values.

Sample

Non-fat Milk Powder (SRM 1549:NIST)
Skim Milk Powder (CRM 063:BCR)

References

- Standard Methods of Analysis for Hygienic Chemists (Annotation), edited by the Pharmaceutical Society of Japan, published by Kanehara & Co., Ltd
- Analysis Manual for the Standard Tables of Food Composition in Japan 5th rev, edited by the Resources Council of the former Science and Technology Agency, published by the Japan Resources Association

■Pretreatment

1g of sample was placed on a platinum dish and incinerated to ash over 12 hours at 550°C using an autoclave. 1mL of nitric acid was added to the incinerated sample to dissolve it. Finally, ultra pure water was added to make 100mL of the sample solution.

■Analytical Conditions

Instrument	: ICPS-8000
High frequency	: 27.12MHz
High frequency output	: 1.2kW
Cooling Gas	: Ar 14.0L/min
Plasma Gas	: Ar l.2L/min
Carrier Gas	: Ar 0.7L/min
Purge Gas	: Ar 3.5L/min
Sample suction rate	: 1.0mL/min
Observation method	: Horizontal
Sample induction	: Coaxial nebulizer

Table 4.17.2 Analysis results for standard powdered milk (wt-%)

Element	NIST-SRM 1549		BCR-CRM 063	
	Quantitative value	Guaranteed value	Quantitative value	Guaranteed value
Na	0.51	0.47 ± 0.03	0.46	0.457 ± 0.016
K	1.68	1.69 ± 0.03	1.76	1.78 ± 0.07
Ca	1.31	1.3 ± 0.03	1.29	1.26 ± 0.03
Mg	0.123	0.120 ± 0.003	0.118	0.112 ± 0.003
P	1.07	1.06 ± 0.2	1.02	1.04 ± 0.03
Fe*	2.3	1.78 ± 0.10	2.6	2.06 ± 0.25
Zn*	47.4	46.1 ± 2.2	43	(42)
Mn*	0.27	0.26 ± 0.06	0.25	(0.226)

*: µg/g ( ) : Reference value

4.18 Analysis of Powdered Milk Using ICPM-8500 - ICP/MS

■Explanation

Trace elements contained in powdered milk were analyzed using the ICPM-8500. A microwave sample decomposition system (sealed type) was used.

■Sample

Commercially available powdered milk

■Elements Analyzed

Al, Cr, Mn, Fe, Ni, Cd, Pb

■Sample preparation

The powdered milk was decomposed with the following method to make the analysis sample.

1) 0.5g of the sample was measured out in a microwave decomposition container and 10mL of nitric acid and 3mL of hydrogen peroxide were added. The mixture was left to stand for approx. 3 hours.
2) After sealing the container, the mixture was processed by the decomposition sequence of the microwave system (approx. 30 minute × twice).
3) The container was left to cool, the decomposed solution was transferred to a fluororesin beaker and then heated on a hot plate.
4) The solution was transferred to a polyethylene container when the volume has been reduced to 2 to 3mL, and adjusted to 50mL with purified water. Y, In and Tl (50ppb for each) were added as internal standard elements.

References

- Standard Tables of Food Composition in Japan Analysis Manual 5th revision (Japan Resources Association)
- Standard Methods of Analysis for Hygienic Chemists Notes (Edited by the Pharmaceutical Society of Japan)

■Calibration curve sample

A metal standard solution (1000ppm) for atomic absorption analysis was mixed and diluted using ultra pure water. 50ppb of internal standard elements and 0.5% of nitric acid were added.

■Instrument and analytical conditions

Table 4.18.1 ICP/MS Analytical conditions

Instrument	:	ICPM-8500
Plasma unit		
High frequency output	:	1.2 (kW)
Coolant gas flow rate (Ar)	:	7 (L/min)
Plasma gas flow rate (Ar)	:	1.5 (L/min)
Carrier gas flow rate (Ar)	:	0.62 (L/min)
Sample induction unit		
Nebulizer	:	Concentric
Sample suction rate	:	0.4 (mL/min)
Chamber	:	Cooled Scott chamber
Plasma torch	:	Triple tube mini-torch
Sampling depth	:	5 (mm)
Sampling interface	:	Cu

■Analysis

Quantitative analysis by the calibration curve method.

■Results

Table 4.18.2 shows the quantitative results.

Table 4.18.2 Results of the Quantitative Analysis of Powdered Milk (µg/g)

Element	M/Z	INT	Quantitative value
Al	27	Y	2.3
Cr	52	Y	0.11
Mn	55	Y	0.28
Fe	57	Y	7.8
Ni	60	Y	0.33
Cd	111	In	0.01
Pb	208	Tl	0.04

4.19 Analysis of Plants Using ICPM-8500 (1) - ICP/MS

■Explanation

Plant reference samples were analyzed using the ICPM-8500. Featuring a wide dynamic range and exceedingly high sensitivity, ICP/MS is an effective method capable of the batch analysis of trace inorganic components such as Pb, Cd, As and Se in the same solution.

■Samples

Rice Flour (NIES No.10)
Tomato Leaves (NIST SRM1573)
Citrus Leaves (NIST SRM1572)

■Elements analyzed

Ni, Pb, As, Hg, Cd, Al, Cr

■Sample preparation: pressurized decomposition

0.1g of the samples were measured out in fluororesin pressurized containers and 1mL of nitric acid was added to each of them. After sealing the containers, they were heated for 2 hours at 170°C. After being let cool, Ho and Rh (10ppb for each) were added as internal standard elements, and they were adjusted to 50mL with ultra pure water to make sample solutions.

■Provision of analysis samples

Tokyo University of Agriculture, Production and Environmental Chemistry Laboratory (Soil Science Laboratory)

References

- 46th notification by the Environment Agency (Environmental quality standards for soil)
- Partial amendment to the Water Pollution Control Law enforcement regulations etc. concerned with environmental standards promulgated on March the 8th 1993
- JIS K0102-1998 (Testing Methods for Industrial Wastewater)

■Calibration curve sample

A standard solution for atomic absorption analysis (1000ppm) was mixed and diluted with ultra pure water. 50ppb of internal standard element and 0.5% of nitric acid were added.

■Instrument and analytical conditions

Table 4.19.1 ICP/MS Analytical conditions

Instrument	:	ICPM-8500
Plasma unit		
High frequency output	:	1.2 (kW)
Coolant gas flow rate (Ar)	:	7 (L/min)
Plasma gas flow rate (Ar)	:	1.5 (L/min)
Carrier gas flow rate (Ar)	:	0.62 (L/min)
Sample induction unit		
Nebulizer	:	Concentric
Sample suction rate	:	0.4 (mL/min)
Chamber	:	Cooled Scott chamber
Plasma torch	:	Triple tube mini-torch
Sampling depth	:	5 (mm)
Sampling interface	:	Cu

■Analysis

Quantitative analysis by the calibration curve method.

■Results

The quantitative results are shown in Table 4.19.2. The measurement results have been multiplied by the dilution factor to express them as concentrations in solids. Results for all three samples showed good accordance with the certified values.
Fig. 4.19.1 shows the calibration curves and Fig. 4.19.2 shows the mass spectra.

4.19 Analysis of Plants Using ICPM-8500 (2) - ICP/MS

Table 4.19.2 Analysis results of plant samples (μg/g)

Sample		Brown Rice NIES No.10		Tomato Leaves NIST SRM1573		Citrus Leaves NIST SRM1572	
Element	M/Z	Quantitative value	Certified value	Quantitative value	Certified value	Quantitative value	Certified value
V	51	0.049	—	0.89	—	0.19	—
Cr	52	0.31	0.22*	3.2	4.5±0.5	0.81	0.8±0.2
Co	59	0.05	0.02*	0.42	0.6*	0.06	0.02*
Ni	60	0.41	0.39±0.04	1.2	—	0.77	0.6±0.3
Cu	63	3.0	3.3±0.2	9.4	11±1	14	16.5±1.0
Zn	66	23.3	22.3±0.9	56	62±6	29	29±2
As	75	0.12	0.11*	0.28	0.27±0.05	3.1	3.1±0.3
Se	82		0.02*	0.09	—	0.04	—
Mo	98	0.45	0.42±0.05	0.49	—	0.12	0.17±0.09
Cd	111	0.29	0.32±0.02	2.6	3*	0.11	0.03±0.01
Pb	208	1.6	—	5.8	6.3±0.3	12.0	13.3±2.4

Figures with "*" are reference values.

4.19 Analysis of Plants Using ICPM-8500 (3) - ICP/MS

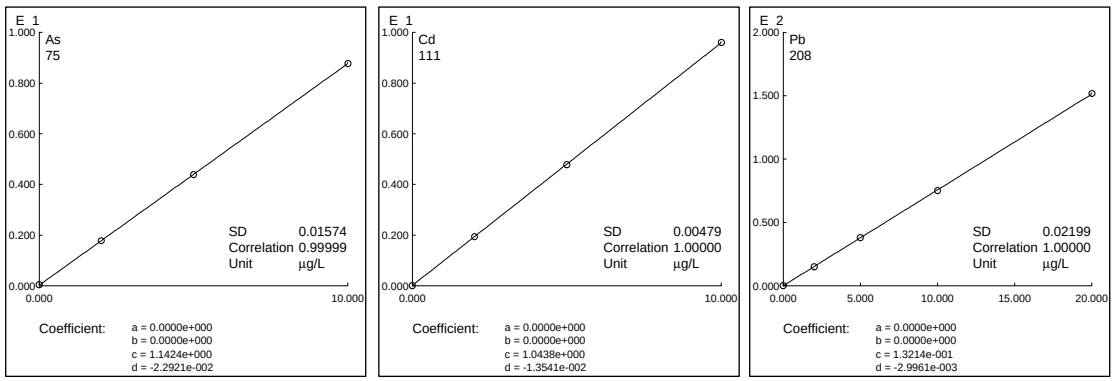


Fig.4.19.1 Calibration Curves for Plant Samples

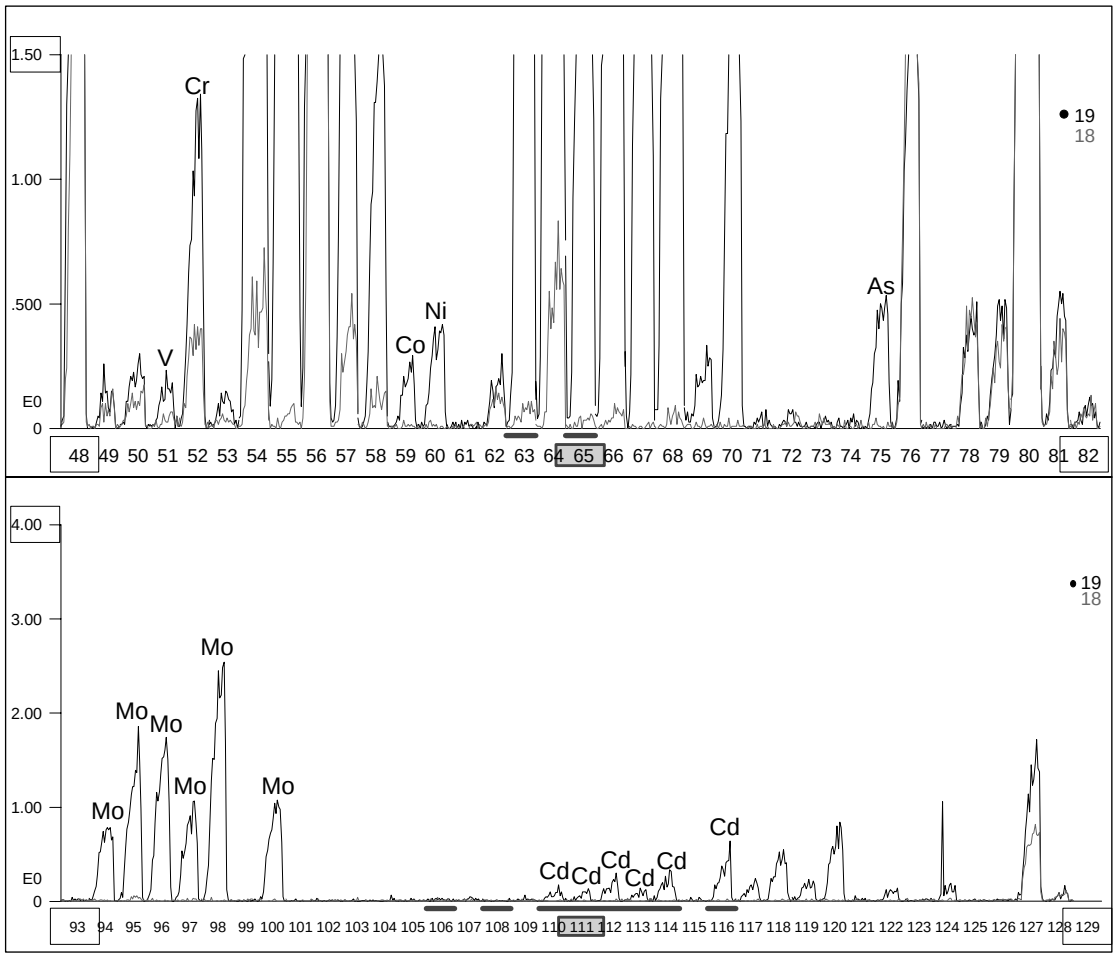


Fig.4.19.2 Mass Spectra for Plant Samples

5.1 Individual and Rapid (Simultaneous) Analysis Methods for Residual Pesticides

Methods for analyzing residual pesticides in foods are stipulated for individual agricultural and marine products, as well as individual pesticides. As of January, 2005, the Japan Food Sanitation Law stipulates 138 different analysis methods for 244 types of pesticides applied to 262 kinds of agricultural products. Meanwhile, to correspond to the increase in the number of items to be analyzed, rapid analysis methods have been developed for the purpose of screening before individual analysis.

■(Individual Analysis Methods) Since the mid 70s

Individual pesticides, or multiple pesticides with relatively similar characteristics grouped together, are analyzed primarily with GC or HPLC after pretreatment such as extraction, concentration and cleaning up from agricultural products. If substances suspected to be pesticides are detected in these tests, GC/MS or LC/MS qualitative analysis is performed for verification. Although these individual analysis methods produce reliable results, they are not effective for handling the increasing number of pesticides due to the time-consuming manual operations required for pretreatment.

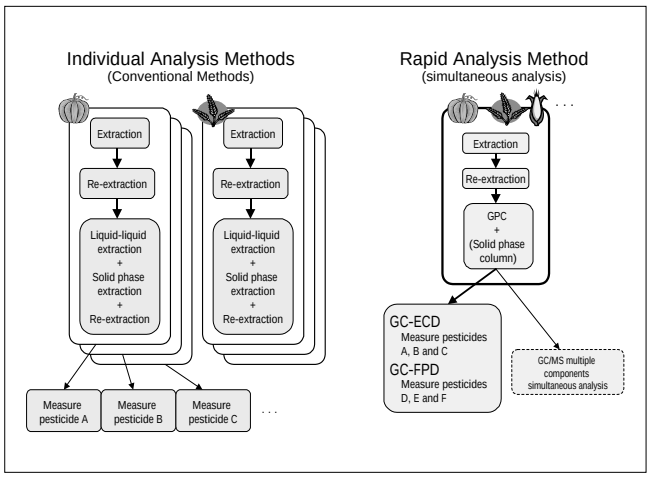


Fig. 5.1.1 Procedures for Individual and Rapid Analysis Methods

■(Rapid Analysis Methods) Since 1997

In order to respond to increasing numbers of imported foods, processed foods and pesticide regulations, screening analysis that provides rapid results is becoming more common. In rapid analysis methods, the procedures of pretreatment such as extraction and cleaning-up is standardized regardless of the type of agricultural product or pesticide, so that multiple substances are simultaneously analyzed using GC, GC/MS or HPLC. Also a portion of pretreatment is automated by the GPC clean-up method. Pesticides are separated into groups, such as halogenated and phosphorous groups, which are analyzed by GC-ECD or GC-FPD.

	Individual Analysis Methods (Conventional Methods)	Rapid Analysis Methods (simultaneous analysis of multiple substances)
(1) Development of simple analysis methods	-Different procedures for different pesticides and products. Difficult to test for all regulated pesticides.	-Applicable to almost all agricultural products. -Simple pretreatment procedures. -Rapid screening with GC/MS.
(2) Reduced usage of harmful organic solvents	-Harmful organic solvents used. -Large amounts used for pretreatment operations and instrument cleaning.	-Non-halogenated solvents are used.
(3) Increased reliability	-Time and labor limitations make controlling accuracy of all analysis procedures difficult. -Extensive manual operations affect analysis quality.	-Reduced variability due to partially automated pretreatment.

Fig. 5.1.2 Comparison of Individual and Rapid Analysis Methods

5.2 List of Analysis Methods for Regulated Residual Pesticides in Agricultural Products (Official Methods) (1)

Use apparatus ○:Qualitative&Quantitative Analysis △:Verification Test
N.D.:Not detected —:Regulation value is not specified

No	Pesticides	Regulation Valie(ppm)					GC				GC/MS	HPLC			LC/MS	Other Methods
		Rice	Soybean	Onion	Welsh Onion	Spinach	ECD	FID	FPD	FTD		UV	Fluorescence	Post-column		
1	2,4-D	0.1	0.05	—	—	—	○				△					
2	Aldrin	—	—	—	—	—	○				△					
3	BHC	0.2	0.2	—	—	0.2	○				△					
4	DDT(DDD.DDE)	0.2	0.2	—	—	0.2	○				△					
5	Dicofol	—	—	—	—	—	○				△					
6	Dieldrin	N.D.	—	—	—	N.D.	○				△					
7	Endrin	N.D.	—	—	—	N.D.	○				△					
8	Fenpropathrin	—	0.1	—	—	—	○				△					
9	Halfenprox	—	—	—	—	—	○				△					
10	Tefluthrin	—	—	—	—	—	○				△					
11	Trifluralin	0.05	0.15	0.05	0.1	0.05	○				△					
12	2,4,5-T	N.D	N.D	N.D	N.D	N.D.	○				△					
13	DCIP	—	—	1.0	1.0	1.0	○				△					
14	Butamifos	0.05	—	0.05	0.05	—			○P	○	△					
15	Cadusafos	—	—	—	—	—			○P	○	△					
16	Chlorfenvinphos	0.05	0.02	0.05	0.3	—			○P	○	△					
17	Chlorpyrifos	0.1	0.5	0.05	0.01	0.01			○P	○	△					
18	Diazinon	0.1	0.1	—	0.1	0.1			○P	○	△					
19	Dimethoate	—	—	—	—	—			○P	○	△					
20	Dimethylvinphos	0.1	—	—	—	—			○P	○	△					
21	Edifenphos	0.2	—	—	—	—			○P	○	△					
22	EPN	0.1	—	—	—	0.1			○P	○	△					
23	Ethoprophos	0.005	0.02	0.02	—	—			○P	○	△					
24	Etrimfos	0.1	—	0.1	0.1	0.2			○P	○	△					
25	Fenitrothion	0.2	0.2	0.2	0.2	0.2			○P	○	△					
26	Fensulfothion	—	0.02	0.1	—	—			○P	○	△					
27	Fenthion	0.05	—	—	—	—			○P	○	△					
28	Fosthiazate	—	—	—	—	—			○P	○	△					
29	Malathion	0.1	0.5	8.0	8.0	2.0			○P	○	△					
30	Parathion	N.D.	0.3	0.3	0.3	0.3			○P	○	△					
31	Parathion-methyl	1.0	0.1	1.0	1.0	1.0			○P	○	△					
32	Phenthoate	0.05	—	—	—	—			○P	○	△					
33	Phosalone	—	—	—	—	—			○P	○	△					
34	Phoxim	0.05	—	0.05	—	—			○P	○	△					
35	Pirimifos-methyl	0.20	—	1.0	1.0	1.0			○P	○	△					
36	Prothiofos	—	0.05	0.1	—	—			○P	○	△					
37	Pyraclofos	—	—	—	—	—			○P	○	△					
38	Quinalphos	—	—	—	—	—			○P	○	△					
39	Terbufos	0.005	—	—	—	—			○P	○	△					
40	Thiometon	0.02	0.02	0.10	0.10	0.10			○P	○	△					
41	Tolclophos-methyl	—	0.5	2.0	2.0	2.0			○P	○	△					
42	Triazophos	N.D.	N.D.	N.D.	—	—			○P	○	△					
43	EPTC	0.1	0.1	0.04	0.04	0.1				○	△					
44	Dicamba	0.05	0.05	—	—	—	○				△					
45	MCPA	0.1	0.1	—	—	—	○				△					
46	Acrinathrin	—	0.1	0.1	—	—	○				△					
47	Bifenthrin	—	—	—	0.5	—	○				△					
48	Cyfluthrin	—	0.2	2.0	2.0	—	○				△					
49	Cyhalothrin	—	0.2	0.5	2.0	0.5	○				△					
50	Cypermethrin	—	0.05	0.1	5.0	2.0	○				△					
51	Deltamethrin	1.0	0.1	0.1	0.1	0.5	○				△					
52	Fenvalerate	—	0.20	0.50	0.50	0.50	○				△					

5.2 List of Analysis Methods for Regulated Residual Pesticides in Agricultural Products (Official Methods) (2)

Use apparatus ○:Qualitative&Quantitative Analysis △:Verification Test
N.D.:Not detected —:Regulation value is not specified

No	Pesticides	Regulation Valie(ppm)					GC				GC/MS	HPLC			LC/MS	Other Methods
		Rice	Soybean	Onion	Welsh Onion	Spinach	ECD	FID	FPD	FTD		UV	Fluorescence	Post-column		
53	Flucythrinate	—	0.1	0.10	—	0.50	○				△					
54	Fluvalinate	—	—	0.1	0.5	—	○				△					
55	Permethrin	2.0	0.05	3.0	3.0	2.0	○				△					
56	Pyrethrins	3	1	1	1	1	○				△					
57	Tralomethrin	—	0.05	0.5	0.5	0.5	○				△					
58	Acibenzolar-S-methyl	0.1	—	—	—	—						○			△	
59	Azimsulfuron	0.1	—	—	—	—						○			△	
60	Flazasulfuron	—	—	—	—	—						○			△	
61	Halosulfuron methyl	0.1	—	—	—	—						○			△	
62	Acequinocyl	—	—	—	—	—						○			△	
63	Acetamiprid	—	—	—	—	—				○	△					
64	Acephate	—	0.5	0.5	0.1	—			○P		△					
65	Methamidophos	—	0.05	—	—	—			○P		△					
66	Azoxystrobin	5	—	0.1	5	—						○			△	
67	Amitraz	—	—	—	—	—				○	△					
68	Amitrole	N.D.	N.D.	N.D.	N.D.	N.D.							○			
69	Alachrol	—	0.2	—	—	0.01				○	△					
70	Bitertanol	—	0.2	—	—	—				○	△					
71	Butachlor	0.1	—	—	—	—				○	△					
72	Diethofencarb	—	0.1	5.0	5.0	5.0				○	△					
73	Fenarimol	—	—	0.5	0.5	0.5				○	△					
74	Fenobucarb	1.0	—	0.3	0.5	1.0					△			○		
75	Flutolanil	2.0	1.0	—	2.0	2.0				○	△					
76	Isoprocarb	0.5	—	—	—	—				○	△					
77	Kresoxim-methyl	—	—	—	2	—				○	△					
78	Lenacil	—	—	0.3	0.3	0.3				○	△					
79	Mefenacet	0.1	—	—	—	—				○	△					
80	Mepronil	2.0	—	—	—	1.0				○	△					
81	Metolachlor	0.1	0.2	1	—	0.3				○	△					
82	Paclobutrazol	0.1	—	—	—	—				○	△					
83	Pretilachlor	0.1	—	—	—	—				○	△					
84	Pyriminobac-methyl	0.1	—	—	—	—				○	△					
85	Pyriproxyfen	—	—	—	—	—				○	△					
86	Tebufenpyrad	—	—	—	—	—				○	△					
87	Thenylchlor	0.1	—	—	—	—				○	△					
88	Aldicarb	0.02	0.02	0.05	—	—					△			○		
89	Bendiocarb	0.02	—	—	—	—					△			○		
90	Carbaryl	1.0	—	—	—	1.0								○		
91	Ethiofencarb	—	1.0	—	—	0.50					△			○		
92	Oxamyl	0.02	0.10	0.05	—	—					△			○		
93	Pirimicarb	—	—	0.50	0.50	1.0					△			○		
94	Isofenphos	—	—	0.10	—	—			○P	○	△					
95	Inabenfide	0.05	—	—	—	—						○			△	
96	Iprodione	3.0	0.2	0.5	5.0	5.0					(△)	○				
97	Imazamox-ammonium	—	0.1	—	—	—						○			△	
98	Imazalil	0.05	—	—	—	—						○				
99	Bensulfuron methyl	0.1	—	—	—	—						○				
100	Imazosulfuron	0.1	—	—	—	—						○				
101	Iminoctadine	0.05	0.03	0.1	0.1	—								○		
102	Imibenconazole	—	—	—	—	—				○	△					
103	Indanofan	0.1	—	—	—	—						○			△	
104	Uniconazole P	0.1	—	—	—	—				○	△					

5.2 List of Analysis Methods for Regulated Residual Pesticides in Agricultural Products (Official Methods) (3)

Use apparatus ○:Qualitative&Quantitative Analysis △:Verification Test
N.D.:Not detected —:Regulation value is not specified

No	Pesticides	Regulation Valie(ppm)					GC				GC/MS	HPLC			LC/MS	Other Methods
		Rice	Soybean	Onion	Welsh Onion	Spinach	ECD	FID	FPD	FTD		UV	Fluorescence	Post-column		
105	Chlorpropham	—	0.20	0.05	—	0.05				○	△					
106	Esprocarb	0.1	—	—	—	—				○	△					
107	Pendimethalin	0.2	0.2	0.2	0.2	—				○	△					
108	Pyributicarb	0.1	—	—	—	—				○	△					
109	Thiobencarb	0.2	0.2	0.2	0.2	0.2				○	△					
110	Etoxazole	—	—	—	—	—				○	△					
111	Ethoxyquin	—	—	—	—	—							○			
112	Ethofenprox	0.5	0.2	—	2	—						○				
113	Etobenzanid	0.1	—	—	—	—						○				
114	Emamectin benzoate	—	—	—	—	—							○		△	
115	Cafenstrole	0.1	—	—	—	—				○	△					
116	Cyproconazole	0.1	—	—	0.2	—				○	△					
117	Difenoconazole	—	0.05	—	—	—				○	△					
118	Fludioxonil	0.02	0.1	0.1	—	—				○	△					
119	Hexaconazole	—	—	—	0.1	—				○	△					
120	Penconazole	—	—	0.1	2	—				○	△					
121	Propiconazole	0.1	0.05	0.05	0.05	0.05				○	△					
122	Simetryn	0.05	—	—	—	—				○	△					
123	Tebuconazole	0.05	—	0.2	—	—				○	△					
124	Tetraconazole	—	—	—	—	—				○	△					
125	Thifluzamide	0.5	—	—	—	—				○	△					
126	Triadimenol	—	—	—	—	—				○	△					
127	Captafol	N.D.	N.D.	N.D.	N.D.	N.D.	○				△					
128	Captan	—	—	—	—	—	○				△					
129	Chlorobenzilate	—	—	—	—	—	○				△					
130	Chlorothalonil	0.1	0.2	0.5	5	—	○				△					
131	Folpet	—	—	2	—	—	○				△					
132	Carpropamid	1	—	—	—	—	○				△					
133	Quizalofop-ethyl	—	0.3	0.05	0.05	0.05						○			△	
134	Chinomethionat	—	—	—	—	—			○S		△					
135	Quinchlorac	5	—	—	—	—						○				
136	Cumyluron	0.1	—	—	—	—						○				
137	Glyphosate	0.1	20	0.2	0.2	0.2							○			
138	Glufosinate	0.50	2.0	0.20	0.20	0.50			○P	○						
139	Clethodim	—	10	0.5	—	—						○			△	
140	Clofentezine	—	0.05	—	—	—				○	△					
141	Chlorimuron ethyl	—	0.05	—	—	—						○				
142	Tribenuron-methyl	—	—	—	—	—						○				
143	Chlorsulfuron	0.05	—	—	—	—						○				
144	Metsulfuron-methyl	0.05	—	—	—	—						○				
145	Bifenox	0.1	—	—	—	—	○				△					
146	Chlorphenapyr	—	—	—	—	—	○				△					
147	Chlorfluazuron	—	1.0	2.0	2.0	2.0						○				
148	Diflubenzuron	—	0.1	0.05	—	—						○				
149	Flufenoxuron	—	—	—	10	—						○				
150	Hexaflumuron	—	—	—	—	—						○				
151	Lufenuron	—	—	—	3	—						○			△	
152	Tebufenozide	0.5	—	—	—	—						○				
153	Teflubenzuron	0.05	0.1	—	1	—						○				
154	Chlormequat	—	—	—	—	—				○						
155	Cyhexatin	N.D.	N.D.	N.D.	N.D.	N.D.			○Sn		△					
156	Fenbutatin oxide	—	—	—	—	—			○Sn		△					

5.2 List of Analysis Methods for Regulated Residual Pesticides in Agricultural Products (Official Methods) (4)

Use apparatus ○:Qualitative&Quantitative Analysis △:Verification Test
N.D.:Not detected —:Regulation value is not specified

No	Pesticides	Regulation Valie(ppm)					GC				GC/MS	HPLC			LC/MS	Other Methods
		Rice	Soybean	Onion	Welsh Onion	Spinach	ECD	FID	FPD	FTD		UV	Fluorescence	Post-column		
157	Cyanazine	—	0.02	0.05	0.05	—				○	△					
158	Diafenthiuron	—	—	—	—	—				○	△	○			△	
159	Cycloxydim	—	2	0.5	0.2	—			○S		△					
160	Cyclosulfamuron	0.1	—	—	—	—						○			△	
161	Dichlofluanid	—	—	0.10	5.0	15	○				△					
162	Diclomezine	2	—	—	—	—						○				
163	Dichlorvos	0.2	0.2	0.1	0.1	0.1			○P	○	△					
164	Trichlorfon	0.20	0.10	0.50	0.50	0.50			○P	○	△					
165	Cyhalofop-butyl	0.1	—	—	—	—				○	△					
166	Dimethenamid	—	0.1	—	—	—				○	△					
167	Difenzoquat	—	—	—	—	—				○	△					
168	Diflufenican	—	0.05	—	—	—				○	△					
169	Cyprodinil	—	0.1	0.05	—	—						○			△	
170	Dimethipin	—	—	—	—	—			○S							
171	Dimethomorph	—	—	0.1	—	—						○			△	
172	Cymoxanil	—	—	2	—	—						○				
173	Bromide	50	—	—	—	—	○				△					
174	Silafluofen	0.5	—	—	—	—						○				
175	Cyromazin	—	—	2	2	7						○				
176	Cinmethylin	0.1	—	—	—	—	○				△					
177	Spinosad	—	0.02	—	—	8						○			△	
178	Sethoxydim	—	10	10	10	10						○				
179	Daimuron	0.1	—	—	—	—					(△)	○				
180	Terbacil	—	—	—	—	—				○	△					
181	Daminozide	N.D.	N.D.	N.D.	N.D.	N.D.				○	△					
182	Tecloftalam	0.2	—	—	—	—	○				△					
183	Desmedipham	—	—	—	—	—						○			△	
184	Copper terephthalate	—	—	—	—	—						○				
185	Trichlamide	—	—	0.2	0.2	0.2	○									
186	Tricyclazole	3	—	—	—	—				○	△					
187	Triflumizole	—	—	1.0	1.0	1.0						○				
188	Lead	—	—	—	—	5.0										Absorptiometry
189	Nitenpyram	0.5	—	—	—	—				○	△	○				
190	Vamidothion	0.2	—	—	—	—	○									
191	Biorethmetrin	1	0.1	0.1	0.1	0.1					○					
192	Picloram	—	—	—	—	—	○				△					
193	Bispyribac-sodium	0.1	—	—	—	—				○	△					
194	Arsenic	—	—	—	—	1.0										Coloration
195	Pymetrozine	0.1	0.02	—	—	—						○			△	
196	Pyrazoxyfen	0.1	—	—	—	—	○				△					
197	Pyraflufen-ethyl	0.1	—	—	—	—				○	△					
198	Pyridaben	—	0.1	—	1.0	—				○	△					
199	Pyridate	—	—	0.2	—	—						○				
200	Pyrifenox	—	—	—	—	—	○				△					
201	Pyrimidifen	—	—	—	—	—				○	△					
202	Pyrimethanil	—	—	—	—	—				○	△					
203	Fipronil	0.01	—	—	—	—	○				△					
204	Fenpyroximate	—	0.1	—	—	2.0						○				
205	Fenhexamid	—	—	0.1	—	—						○			△	
206	Butylate	—	—	—	—	—				○	△					
207	Furametpyr	1	—	—	—	—				○	△	○				
208	Fluazifop	—	1	0.5	0.1	—				○	△					

5.2 List of Analysis Methods for Regulated Residual Pesticides in Agricultural Products (Official Methods) (5)

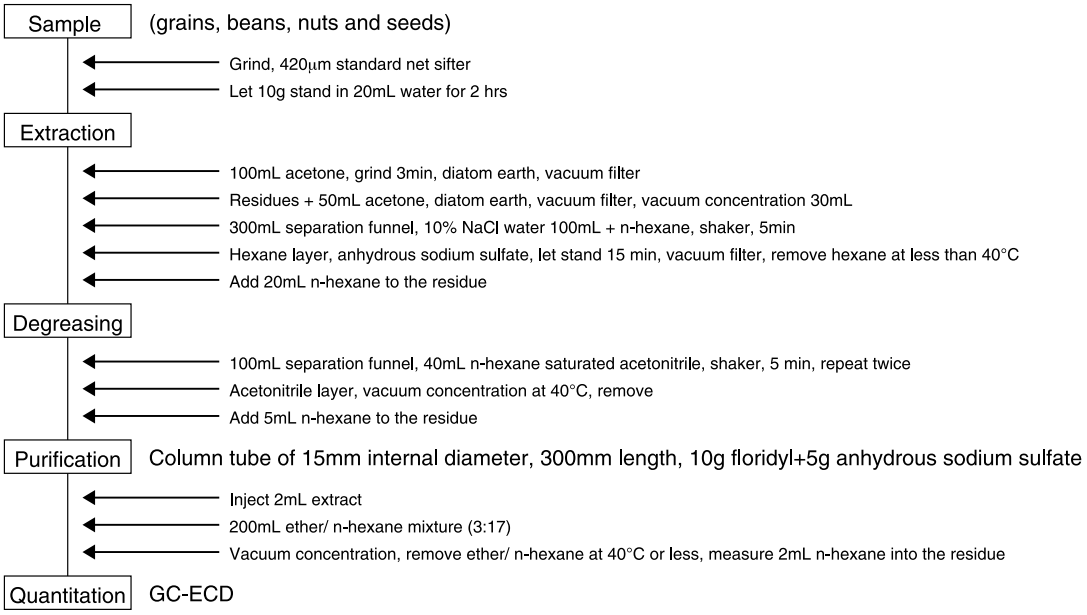
Use apparatus ○:Qualitative&Quantitative Analysis △:Verification Test
N.D.:Not detected —:Regulation value is not specified

No	Pesticides	Regulation Valie(ppm)					GC				GC/MS	HPLC			LC/MS	Other Methods
		Rice	Soybean	Onion	Welsh Onion	Spinach	ECD	FID	FPD	FTD		UV	Fluorescence	Post-column		
209	Fluoroimide	—	—	1	—	—				○	△					
210	Flusilazole	—	—	—	—	—				○	△					
211	Flusulfamide	—	—	—	—	—	○				△					
212	Prochloraz	—	—	—	—	—	○				△					
213	Procymidone	—	2	0.5	5	—	○				△					
214	Propamocarb	0.1	—	—	3.0	10				○	△					
215	Prohexadione Ca	0.2	—	—	—	—						○				
216	Hexythiazox	—	0.5	—	—	—						○				
217	Pencycuron	0.5	—	—	—	1						○				
218	Bentazone	0.2	0.05	0.2	0.05	0.05	○			○						
219	Pentoxazone	0.1	—	—	—	—						○			△	
220	Benfuresate	0.1	—	—	—	—			○S		△					
221	Fosetyl	—	—	50	100	100			○P	○	△					
222	Maleic Hydrazide	—	—	20	25	25				○	△					
223	Myclobutanil	—	—	1.0	1.0	1.0				○	△					
224	Methabenzthiazuron	0.05	0.05	0.05	0.05	—					(△)	○				
225	Methiocarb	0.05	0.05	0.05	0.05	0.05					(△)			○		
226	Methoprene	5.0	—	—	—	—		○								
227	Metribuzin	0.05	0.1	0.5	0.5	0.5	○			○	△					
228	Mepanipyrim	—	—	—	—	—						○				
229	Molinate	0.1	—	—	—	—				○	△					
230	Ethychlozate	—	—	—	—	—						○			△	
231	Oxaziclomefone	0.1	—	—	e	—				○	△					
232	Fenoxanil	1	—	—	—	—				○	△					
233	Dichlocymet	0.5	—	—	—	—				○	△					
234	Tepraloxydim	—	6	0.5	—	—					○					
235	Trinexapac-ethyl	0.5	—	—	—	—						○			△	
236	Famoxadone	—	0.2	0.5	—	—						○			△	
237	Fenoxaprop-ethyl	0.05	0.1	0.1	—	—						○			△	
238	Fentrazamide	0.1	—	—	—	—				○	△					
239	Fluazinam	—	—	0.1	0.1	—	○				△					
240	Flumioxazin	—	0.02	—	—	—				○	△					
241	Novaluron	—	—	—	—	—						○			△	
242	Pyridalyl	—	—	—	5	—	○			○	○					
243	Ethiprole	0.2	—	—	—	—						○			○	
244	Boscalid	—	0.1	3.0	3.0	—				○	○					

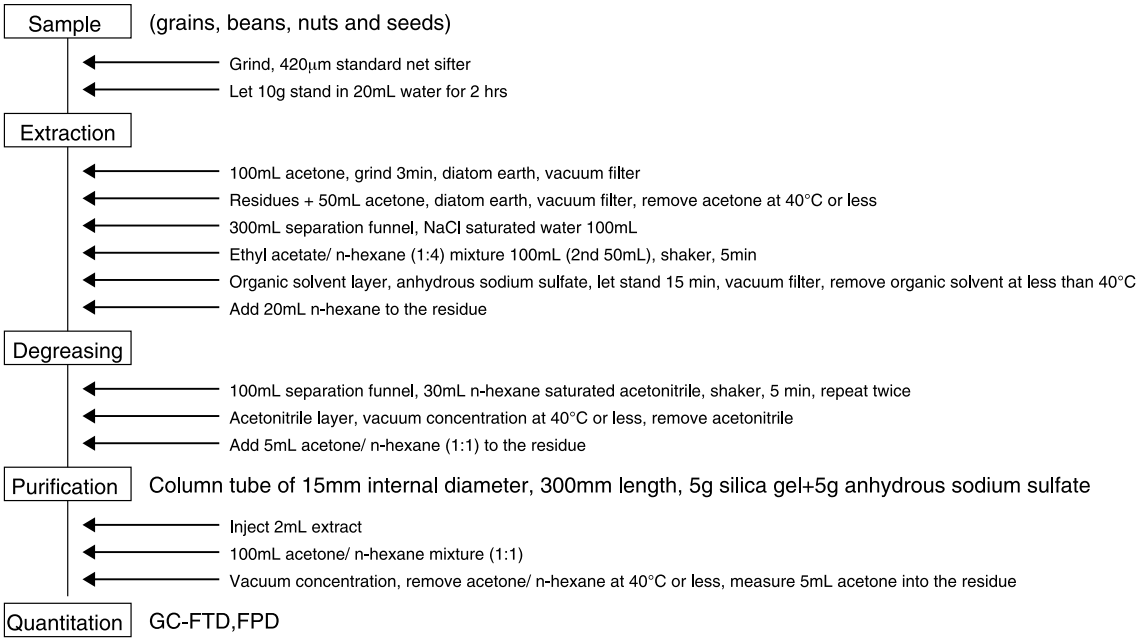
*cadmium, cyanogen, and those compounds are not listed here.

5.3 Sample Pretreatment Example for Residual Pesticide Analysis (1)

(1) Organic chlorine agrichemicals (10 types including BHC, DDT, aldrin, endrin, dicofol, dieldrin, tefluthrin, trifluralin, halfenprox and fenpropathrin)

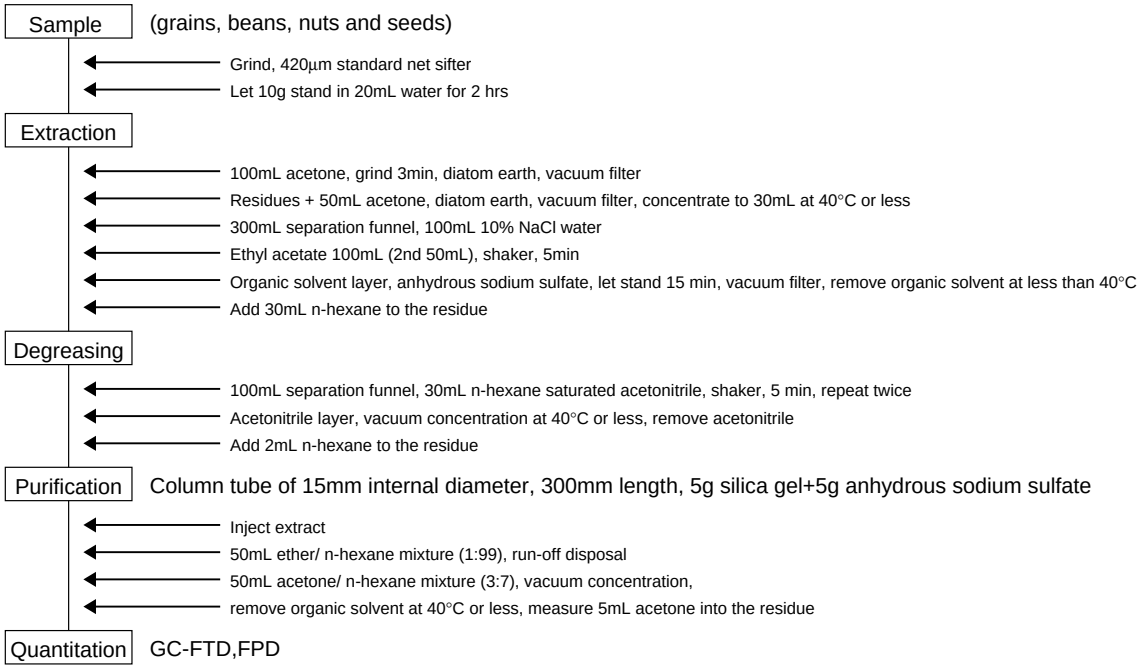


(2) Organophosphorus agrichemicals (29 types including EPN, edifenphos, ethoprophos, etrimfos, cadusafos, quinalphos, chlorpyrifos, chlorfenvinphos, dimethylvinphos, dimethoate, diazinon, thiometon, terbufos, triazophos, tolclophos-methyl, parathion, parathion-methyl, pyraclofos, pirimifos-methyl, fenitrothion (MEP), fensulfothion, fenthion, phenthoate, butamifos, prothiofos, phoxim, phosalone, fosthiazate and malathion)

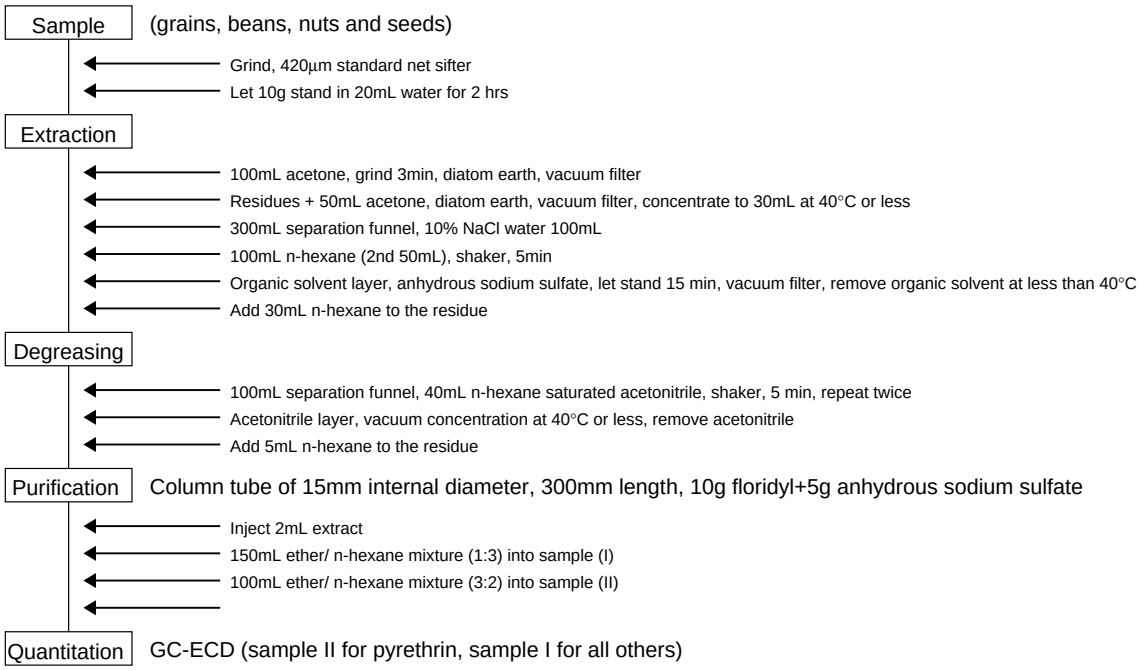


5.3 Sample Pretreatment Example for Residual Pesticide Analysis (2)

(3) Organonitrogen agrichemicals (18 types including alachrol, isoprocarb, kresoxim-methyl, diethofencarb, thenylchlor, tebufenpyrad, paclobutrazol, bitertanol, pyriproxyfen, pyriminobac-methyl, fenarimol, flutolanil, butachlor, pretilachlor, metolachlor, mefenacet, mepronil, lenacil)

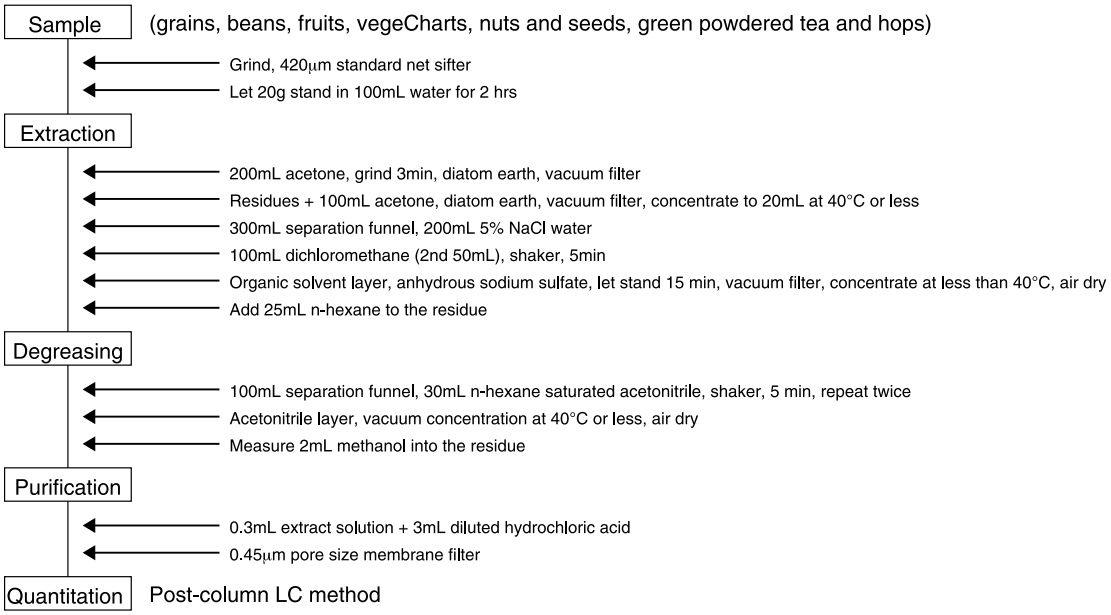


(4) Pyrethroid agrichemicals (12 types including acrinathrin, cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, tralomethrin, bifenthrin, pyrethrin, fenvalerate, flucythrinate, fluvalinate, permethrin)



5.3 Sample Pretreatment Example for Residual Pesticide Analysis (3)

(5) Carbamate-based agrichemicals (7 types including aldicarb, ethiofencarb, oxamyl, carbaryl, pirimicarb, fenobucarb and bendiocarb)



5.4 Residual Veterinary Drug Regulation Values and Analytical Methods

Veterinary drug	Application	ADI(*1)	Residual limit value *6 (ppm)	Qualitative/quantitative analysis methods	Verification test method
Albendazole	Antiparasitic agent	50	0.10~5.0	LC-fluorescence	
Isometamidium	Antiparasitic agent	100	0.1~1.0	LC-UV	
Ivermectin	Antiparasitic agent	1	0.015~0.10	LC-UV	LC/MS
Oxytetracycline	Antibiotic	3	0.01~0.6	LC-UV	
Oxytetracycline/ chlortetracycline/ tetracycline	Antibiotic	30 (*2)	0.1~1.2	LC-fluorescence	LC/MS
Carbadox	Synthetic antibacterial agent	(*3)	0.005~0.030	LC-UV	
Closantel	Antiparasitic agent	25	1.0~5.0	LC-UV	LC-PDA
Gentamycin	Antibiotic	20	0.1~5.0	LC/MS	
Sarafloxacin	Synthetic antibacterial agent	0.3	0.01~0.08	LC-fluorescenceLC/MS	
Diclazuril	Anticoccidial agent	30	0.5~3.0	LC-UV	LC-PDA, LC/MS
Cyromazine	Antiparasitic agent	18	0.01~0.20	LC-UV	LC-PDA, LC/MS
Streptomycin/ dihydrostreptomycin	Antibiotic	50 (*5)	0.2~1.0	LC/MS	
Spectinomycin	Antibiotic	40	0.2~5.0	LC/MS	
Spiramycin	Antibiotic	50	0.2~0.6	LC-UV Pork:L-cysteine conjugation-> bioassay measurement using paper disk	LC/MS
Sulfadimidine	Synthetic antibacterial agent	50	0.025~0.10	LC-UV	
Zeranol	Hormone	0.5	0.002, 0.01	LC-UV	
Danofloxacin	Synthetic antibacterial agent	18	0.05~0.40	LC-fluorescence LC/MS	
Thiabendazole	Antiparasitic agent	100	0.10	LC-UV	LC/MS
Triclabenzadole	Antiparasitic agent	2.7	0.10~0.30	LC-UV	
Trenbolone	Hormone	0.02	0.002, 0.01	LC-UV	
Nicarbazin	Antibiotic	400	0.2	LC-UV	LC-PDA, LC/MS
Neomycin	Antibiotic	60	0.5~10.0	LC/MS	
Flubendazole	Antiparasitic agent	12	0.01~0.5	LC-UV	LC-PDA
Benzylpenicillin	Antibiotic	(*4)	0.004~0.05	Culture media method using bacillus stearothermophilus	TLC
Moxidectin	Antiparasitic agent	1.5	0.2~0.5	LC-UV	LC/MS
Eprinomectin	Antiparasitic agent	10	0.02~2.00	LC-fluorescence	LC/MS
Tilmicosin	Antibiotic	40	0.05~1.5	LC-UV	LC-PDA, LC/MS
Ceftiofur	Antibiotic	50	0.1~6.0	LC-UV	LC-PDA, LC/MS
Levamisole	Antiparasitic agent	6	0.01~0.1	LC-UV	LC-PDA, LC/MS

(*1) Unit: μg/kg body weight per day
(*2) Independent value or total value.
(*3) No value set for carbadox.
(*4) The amount of benzylpenicillin ingested orally should not exceed 30 μg per person per day.
(*5) Total value.
(*6) The residual limit values vary with the type of meat (beef, pork, etc.) and the part (liver, kidney, etc.) and so the values are indicated as a range.

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