（半）発酵茶ではカラム精製において過負荷となり、夾雑成分の除去が不十分となることが判明した。そこで本研究では、通知I法を基に、より簡便かつ効果的に夾雑成分を除去する方法を検討し、煎茶、烏龍茶、紅茶および抹茶に適用可能な残留農薬一斉分析法を開発したので報告する。なお、抹茶以外の茶（茶葉）の規格基準への適否判定においては、茶葉から有機溶媒等で直接抽出し、茶葉中の濃度により判定する農薬と、茶葉を熱湯で浸出し、その浸出液中の濃度により判定する農薬があるが、本検討ではいずれの農薬も対象とした。

II 実験方法

1. 試料

市販の煎茶、烏龍茶、紅茶および抹茶を用いた。煎茶、烏龍茶および紅茶は、遠心粉砕機で粉砕して均一化し、425 μm の標準網ふるいに通したものを用いた。抹茶は均一化したものを用いた。

2. 試薬・試液

試験溶液の調製に用いたアセトニトリル、トルエンおよびメタノールは関東化学（株）製の残留農薬試験用試薬、水は超高純度蒸留水精製装置で蒸留したものを用いた。移動相溶媒は、関東化学（株）製の LC-MS 用蒸留水およびメタノールを用いた。

塩化ナトリウムは、和光純薬工業（株）製の残留農薬試験用試薬を用いた。酢酸アンモニウム、リン酸水素二カリウムおよびリン酸二水素カリウムは、和光純薬工業（株）製の特級を用いた。ろ紙は桐山製作所製 No.5B、ケイソウ土は和光純薬工業（株）製のセライト 545 を用いた。

各農薬標準品は、林純薬工業（株）、関東化学（株）、和光純薬工業（株）、Dr. Ehrenstorfers 社および Riedel-de Haën 社の残留農薬試験用試薬を用いた。標準原液（1000 mg/L）は、各農薬 10 mg を精秤し、アセトニトリル（アセトニトリルへの溶解性が低い場合はメタノール）10 mL に溶解して調製した。添加回収試験用の混合標準溶液は、各農薬の標準原液を混合し、アセトニトリルで適宜希釈して調製した。検量線作成用の混合標準溶液は、添加回収試験用の混合標準溶液をメタノールで適宜希釈して用時調製した。

リン酸緩衝液（0.5 mol/L、pH7.0）は、以下の通りに調製した。リン酸水素二カリウム（K_2HPO_4）52.7 g およびリン酸二水素カリウム（KH_2PO_4）30.2 g を量り採り、水約 500 mL に溶解し、1 mol/L 水酸化ナトリウムまたは 1 mol/L 塩酸を用いて pH を 7.0 に調整した後、水を加えて 1 L とした。

3. 精製ミニカラム

ODS ミニカラムは、Agilent 社製の Mega Bond Elut C18（充てん量 1000 mg）を用いた。グラファイトカーボン /PSA 積層ミニカラムは、ジーエルサイエンス（株）製の InertSep GC/PSA（充てん量 500 mg/500 mg）を用いた。

4. 装置

LC-MS/MS は、Waters 社製の液体クロマトグラフ Alliance 2695 および同社製質量分析計 Micromass Quattro Premier を使用した。遠心粉砕機は Retsch 社製 ZM200、ホモジナイザーは Kinematica 社製 Polytron PT 10-35 GT を用いた。蒸留水精製装置は、藤原製作所（株）製の超高純度蒸留水精製装置 NZJ-2DSYW を用いた。pH メーターは、（株）堀場製作所製 F-52 を用いた。

5. LC-MS/MS 測定条件

1) LC 条件

カラム：Inertsil ODS-4（内径 2.1 mm、長さ 150 mm、粒子径 3 μm、ジーエルサイエンス社製）、ガードカラム：Inertsil ODS-4（内径 1.5 mm、長さ 10 mm、粒子径 3 μm、ジーエルサイエンス社製）、カラム温度：40℃、注入量：5 μL、移動相：5 mmol/L 酢酸アンモニウム溶液（A 液）および 5 mmol/L 酢酸アンモニウム・メタノール溶液（B 液）、移動相流速：0.20 mL/min、グラジエント条件：0 分（A:B = 85 : 15）→ 1 分（A:B = 60 : 40）→ 3.5 分（A:B = 60 : 40）→ 6 分（A:B = 50 : 50）→ 8 分（A:B = 45 : 55）→ 17.5 分（A:B = 5 : 95）→ 33 分（A:B = 5 : 95）→ 33.1 分（A:B = 0 : 100）→ 43 分（A:B = 0 : 100）→ 43.1 分（A:B = 85 : 15）→ 55 分（A:B = 85 : 15）、保持時間：Table 1 に示した。

2) MS 条件

イオン化モード：エレクトロスプレーイオン化法ポジティブモード（ESI (+)）およびネガティブモード（ESI (−)）、測定モード：SRM (selected reaction monitoring)、キャピラリー電圧：3 kV、ソース温度：120℃、コーンガス流量：50 L/h（N_2）、脱溶媒温度：400℃、脱溶媒ガス流量：800 L/h（N_2）、コリジョンガス流量：3.1 × 10^{-3} mbar (Ar)、測定イオン（*m/z*）：Table 1 に示した。

6. 試験溶液の調製

試験溶液の調製方法の概略を Fig. 1 に示した。

1) 抽出

試料 5.00 g に水 20 mL を加え、30 分間放置した。これにアセトニトリル 50 mL を加え、約 1 分間ホモジナイズした後、ケイソウ土を約 1 cm の厚さに敷いたろ紙を用いて吸引ろ過した。残留物を採り、アセトニトリル 20 mL を加え、上記と同様にホモジナイズした後、吸引ろ過した。得られたろ液を合わせ、アセトニトリルを加えて正確に 100 mL とした。

抽出液 5 mL（試料 0.25 g 相当）を採り、アセトニトリル 15 mL を加え、さらに塩化ナトリウム 10 g、リン酸緩衝液（0.5 mol/L、pH 7.0）20 mL を加えて 10 分間振とう後、毎分 3,000 回転で 5 分間遠心分離を行った。

ODS ミニカラム（1000 mg）にアセトニトリル 10 mL を注入し、流出液は捨てた。このカラムに上記のアセトニトリル層を注入し、さらにアセトニトリル 5 mL を注入した。全溶出液を採り、40℃以下で約 1 mL まで減圧濃縮後、窒素気流により溶媒を除去し、残留物をアセトニトリル / トルエン（3 : 1）2 mL を加え、よく混合した。（必要に応じて超音波処理

Table 1. LC-MS/MS parameters for the tested pesticides

	Retention time (min)	Ionization mode	Quantitation				Confirmation			
			Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Acetamiprid	10.8	ESI (+)	223	126	30	24	223	90	30	36
Acibenzolar-*S*-methyl	20.4	ESI (+)	211	136	30	30	211	91	30	18
Acrinathrin	24.4	ESI (+)	559	208	20	12	559	181	20	30
Aldicarb	13.5	ESI (+)	208	116	10	5	208	88	10	13
Aldoxycarb	7.2	ESI (+)	240	148	18	13	240	85	18	21
Anilofos	21.6	ESI (+)	368	199	20	12	368	125	20	30
Aramite	23.4	ESI (+)	352	191	20	12	352	57	20	30
Atrazine	17.6	ESI (+)	216	174	30	18	216	96	30	24
Avermectin B1a	25.0	ESI (+)	891	305	20	28	891	567	20	14
Azamethiphos	14.4	ESI (+)	325	183	20	18	325	112	20	36
Azinphos methyl	19.1	ESI (+)	318	132	10	12	318	160	10	6
Azoxystrobin	19.4	ESI (+)	404	372	26	13	404	329	26	29
Bendiocarb	15.6	ESI (+)	224	167	26	13	224	109	26	21
Benzofenap	23.2	ESI (+)	431	105	34	37	431	119	34	21
Boscalid	19.5	ESI (+)	343	307	34	21	343	271	34	29
Buprofezin	23.2	ESI (+)	306	201	20	12	306	116	20	18
Butafenacil	20.4	ESI (+)	492	331	26	21	492	349	26	13
Carbaryl	16.5	ESI (+)	202	145	26	13	202	127	26	29
Carbofuran	15.6	ESI (+)	222	165	26	13	222	123	26	21
Carpropamid	21.7	ESI (+)	334	139	26	21	334	103	26	45
Chlorfluazuron	24.3	ESI (+)	540	383	30	18	540	158	30	18
Chloridazon	11.4	ESI (+)	222	104	40	24	222	92	40	24
Chloroxuron	20.3	ESI (+)	291	164	34	21	291	72	34	21
Chlorpyrifos	23.8	ESI (+)	352	97	20	36	352	200	20	18
Chlorpyrifos methyl	22.5	ESI (+)	324	125	30	18	324	292	30	18
Chromafenozide	20.6	ESI (+)	395	175	18	13	395	91	18	61
Clofentezine	22.9	ESI (+)	303	138	26	13	303	102	26	37
Clomeprop	23.4	ESI (+)	324	120	26	21	324	203	26	13
Cloquintocet mexyl	23.6	ESI (+)	336	238	26	13	336	192	26	29
Clothianidin	10.0	ESI (+)	250	169	26	13	250	132	26	13
Cumyluron	20.2	ESI (+)	303	185	26	13	303	125	26	37
Cyazofamid	20.9	ESI (+)	325	108	20	12	325	217	20	18
Cycloprothrin	24.2	ESI (+)	499	181	18	37	499	229	18	21
Cyflufenamid	22.1	ESI (+)	413	203	30	36	413	295	30	18
Cyprodinil	22.4	ESI (+)	226	92	42	29	226	107	42	29
Daimuron	20.1	ESI (+)	269	151	26	13	269	91	26	37
Di-allate	23.0	ESI (+)	270	86	30	18	270	109	30	30
Diazinon	21.9	ESI (+)	305	169	30	24	305	153	30	18
Difenoconazol	21.4, 22.2	ESI (+)	406	251	40	24	406	188	40	42
Diflubenzuron	21.1	ESI (+)	311	158	26	13	311	141	26	29
Dimethirimol	17.3	ESI (+)	210	71	40	30	210	98	40	24
Dimethomorph	19.4, 19.8	ESI (+)	388	301	34	21	388	165	34	29
Diuron	18.1	ESI (+)	233	72	34	21	233	160	34	29
Epoxiconazole	20.8	ESI (+)	330	121	30	18	330	101	30	48
Ethion	23.4	ESI (+)	385	199	20	12	385	143	20	24
Ethiprole	18.7	ESI (-)	395	331	20	12	395	250	20	24
Ethofenprox	26.2	ESI (+)	394	177	20	18	394	107	20	42
Etoxazole	24.0	ESI (+)	360	141	40	30	360	304	40	18
Fenamidone	19.5	ESI (+)	312	236	26	13	312	92	26	29
Fenbuconazole	20.3	ESI (+)	337	70	30	18	337	125	30	30
Fenobucarb	19.0	ESI (+)	208	95	20	18	208	152	20	6
Fenoxaprop ethyl	23.1	ESI (+)	362	288	30	18	362	119	30	24
Fenoxycarb	21.3	ESI (+)	302	88	20	18	302	116	20	12
Fenpropathrin	24.0	ESI (+)	350	125	20	18	350	97	20	36
Fenpyroximate (*E*)	25.0	ESI (+)	422	366	26	13	422	215	26	29
Fenpyroximate (*Z*)	23.9	ESI (+)	422	366	26	13	422	215	26	29
Ferimzone	19.9, 20.2	ESI (+)	255	91	40	30	255	132	40	18
Flufenacet	20.6	ESI (+)	364	152	20	18	364	194	20	12
Flufenoxuron	23.9	ESI (+)	489	158	30	18	489	141	30	48
Fluridone	19.2	ESI (+)	330	310	60	30	330	259	60	48
Furametpyr	17.6	ESI (+)	334	157	30	36	334	290	30	18
Furathiocarb	23.3	ESI (+)	383	195	26	21	383	252	26	13
Halfenprox	26.7	ESI (+)	496	183	20	18	496	461	20	12
Hexaflumuron	22.7	ESI (-)	459	439	20	12	459	175	20	42
Hexythiazox	24.0	ESI (+)	353	228	26	13	353	168	26	29
Imazalil	21.7	ESI (+)	297	159	40	24	297	69	40	18
Imibenconazole	23.0	ESI (+)	413	125	40	36	413	127	40	36
Imidacloprid	9.8	ESI (+)	256	209	26	13	256	175	26	21

Table 1. LC-MS/MS parameters for the tested pesticides (continued)

	Retention time (min)	Ionization mode	Quantitation				Confirmation			
			Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Indanofan	21.0	ESI (+)	341	187	18	13	341	175	18	13
Indoxacarb	22.5	ESI (+)	528	203	34	45	528	150	34	21
Iprovalicarb	20.5	ESI (+)	321	119	20	18	321	203	20	6
Isoxaflutole	18.0	ESI (+)	360	251	34	13	360	220	34	37
Isoxathion	22.1	ESI (+)	314	105	20	12	314	97	20	36
Kresoxim methyl	21.3	ESI (+)	314	206	20	6	314	116	20	12
Lactofen	23.2	ESI (+)	479	344	20	12	479	223	20	36
Linuron	19.5	ESI (+)	249	160	26	21	249	182	26	13
Lufenuron	23.6	ESI (-)	509	326	26	21	509	175	26	37
Malathion	19.6	ESI (+)	331	127	20	12	331	99	20	24
Mepanipyrim	21.3	ESI (+)	224	106	50	24	224	77	50	36
Methabenzthiazuron	18.0	ESI (+)	222	165	30	18	222	150	30	30
Methamidophos	3.8	ESI (+)	142	94	20	12	142	125	20	12
Methidathion	18.3	ESI (+)	303	145	20	12	303	85	20	18
Methiocarb	19.5	ESI (+)	226	169	20	12	226	121	20	18
Methomyl	8.3	ESI (+)	163	106	10	13	163	88	10	13
Methoxyfenozide	20.1	ESI (+)	369	149	18	21	369	91	18	45
Monocrotophos	8.3	ESI (+)	224	193	20	6	224	127	20	18
Monolinuron	17.0	ESI (+)	215	126	26	21	215	148	26	13
Myclobutanil	19.4	ESI (+)	289	70	30	18	289	125	30	36
Naproanilide	21.4	ESI (+)	292	171	20	12	292	120	20	24
Nitenpyram	7.2	ESI (+)	271	126	20	30	271	130	20	12
Novaluron	22.7	ESI (+)	493	158	34	21	493	141	34	45
Oxamyl	7.6	ESI (+)	237	90	10	6	237	72	10	12
Oxaziclomefone	23.2	ESI (+)	376	190	18	13	376	161	18	29
Oxycarboxine	11.9	ESI (+)	268	175	20	12	268	147	20	18
Pencycuron	22.4	ESI (+)	329	125	34	29	329	89	34	61
Pentoxazone	23.3	ESI (+)	354	286	34	13	354	186	34	29
Phenmedipham	18.6	ESI (+)	318	168	20	12	318	136	20	24
Phosalone	21.7	ESI (+)	368	182	20	18	368	111	20	36
Pirimicarb	17.6	ESI (+)	239	72	30	18	239	182	30	18
Prochloraz	21.7	ESI (+)	376	308	20	12	376	70	20	24
Profenofos	22.8	ESI (+)	375	305	30	18	375	347	30	12
Propaquizafop	23.5	ESI (+)	444	100	30	18	444	70	30	36
Propargite	23.7	ESI (+)	368	231	20	12	368	175	20	18
Propiconazole	21.3	ESI (+)	342	159	30	24	342	69	30	24
Propoxur	14.9	ESI (+)	210	111	20	12	210	168	20	6
Pyraclostrobin	22.3	ESI (+)	388	194	20	12	388	163	20	24
Pyrazolynate	22.5	ESI (+)	439	91	40	36	439	173	40	18
Pyrazophos	22.2	ESI (+)	374	222	30	24	374	194	30	30
Pyridaben	24.7	ESI (+)	365	147	20	24	365	309	20	12
Pyriftalid	19.4	ESI (+)	319	139	50	30	319	83	50	42
Pyrimidifen	24.2	ESI (+)	378	184	40	24	378	150	40	36
Pyriproxyfen	23.7	ESI (+)	322	96	20	18	322	185	20	24
Quinalphos	21.5	ESI (+)	299	163	30	18	299	147	30	18
Quizalofop ethyl	23.3	ESI (+)	373	299	30	18	373	91	30	30
Simeconazole	20.4	ESI (+)	294	70	26	21	294	73	26	29
Spinosyn A	26.4	ESI (+)	733	142	42	29	733	98	42	61
Spinosyn D	27.2	ESI (+)	747	142	50	29	747	98	50	53
Spiromesifen	23.7	ESI (+)	388	273	10	12	388	255	10	30
Tebuconazole	20.9	ESI (+)	308	70	30	24	308	125	30	36
Tebufenozide	21.2	ESI (+)	353	133	10	18	353	297	10	6
Tebuthiuron	15.9	ESI (+)	229	172	30	18	229	116	30	24
Teflubenzuron	23.6	ESI (+)	381	158	26	13	381	141	26	37
Tetrachlorvinphos	21.3	ESI (+)	367	127	30	12	367	206	30	36
Tetraconazole	20.0	ESI (+)	372	159	40	30	372	70	40	24
Thiabendazole	15.1	ESI (+)	202	175	50	29	202	131	50	29
Thiacloprid	12.4	ESI (+)	253	126	34	21	253	90	34	37
Thiamethoxam	8.2	ESI (+)	292	211	26	13	292	181	26	21
Triadimefon	19.6	ESI (+)	294	69	30	24	294	197	30	18
Triadimenol	19.6	ESI (+)	296	70	10	12	296	99	10	12
Tridemorph	27.7, 28.9	ESI (+)	298	130	50	24	298	57	50	30
Trifloxystrobin	22.4	ESI (+)	409	186	20	18	409	145	20	48
Triflumizole	22.4	ESI (-)	344	276	20	12	344	301	20	12
Triflumuron	20.4	ESI (+)	359	156	26	13	359	139	26	37
Triticonazole	20.6	ESI (+)	318	70	20	12	318	125	20	36
XMC	16.4	ESI (+)	180	123	20	12	180	108	20	24

を行った。）

2）精製

グラファイトカーボン/PSA 積層ミニカラム（500 mg/500 mg）にアセトニトリル/トルエン（3：1）を 10 mL 注入し、流出液は捨てた。このカラムに 1）で得られた溶液を注入した後、アセトニトリル/トルエン（3：1）20 mL（うち 2 mL で 3 回容器を洗浄した）を注入した。全溶出液を 40℃以下で約 1 mL まで減圧濃縮後、窒素気流により溶媒を除去し、残留物をメタノール 1 mL に溶解したものを試験溶液（試料 0.25 g 相当 /mL）とした。

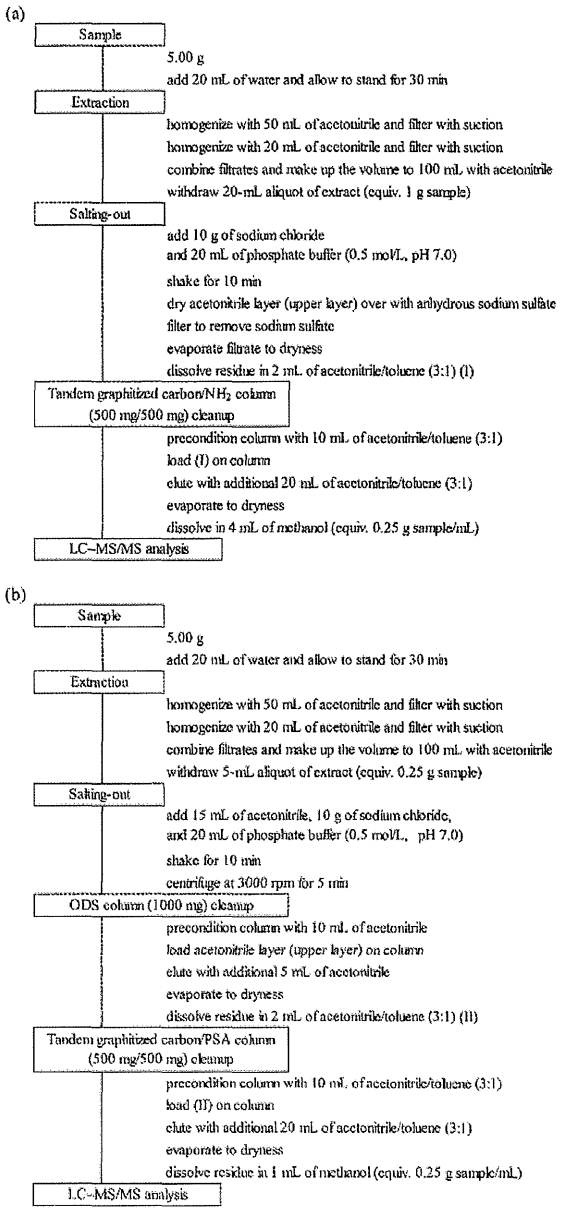

Fig. 1. Flow chart showing the sequence of steps in (a) the Japanese official multiresidue method I for tea, and (b) the modified multiresidue method

7. 添加回収試験

市販の茶を用いて基準値濃度（基準値が設定されていない農薬は 0.01 ppm）で 5 併行の添加回収試験を行った。添加回収試験は、基準値が 1 ppm 以下の農薬と 1 ppm を超える農薬の 2 つのグループに分け、それぞれについて行った。基準値が 1 ppm を超える農薬の添加回収試験においては、6 で得られた溶液 0.5 mL を採り、メタノールで正確に 25 mL としたものを試験溶液（試料 0.005 g 相当 /mL）とした。なお、抽出操作は農薬添加 30 分後に開始した。

8. 定量

基準値の 0.125、0.25、0.5、0.75、1 および 1.5 倍相当濃度（添加回収試験における回収率 12.5、25、50、75、100 および 150％相当濃度）の標準溶液をメタノールで調製し、それぞれ 5 μL を LC-MS/MS に注入して、ピーク面積法で検量線を作成した。試験溶液 5 μL を LC-MS/MS に注入し、検量線から絶対検量線法により濃度を求めた。

9. 試料マトリックスの測定への影響

ブランク試験溶液（農薬の残留していないブランク試料を用い、本法に従って調製した試験溶液）100 μL をバイアルに採り、窒素を吹き付けて乾固した後、残留物を添加回収試験における回収率 100％相当濃度の溶媒標準溶液（溶媒で調製した標準溶液）100 μL に溶解してマトリックス標準溶液とした。マトリックス標準溶液と溶媒標準溶液を交互に各 2 回測定し、溶媒標準溶液のピーク面積の平均値に対するマトリックス標準溶液のピーク面積の平均値の比を求めて試料マトリックスの測定への影響を評価した。

Ⅲ　結果および考察

検討には既報[4]で用いた 135 化合物を用いた。LC-MS/MS 測定は、通知 I 法[1]に示された条件で行った。また、既報[4]と同様に、保持時間が最も長いトリデモルフが溶出した後、B 液（5 mmol/L 酢酸アンモニウム・メタノール溶液）100％で 10 分間のカラム洗浄を行った。

1. 試験溶液調製方法の検討

1）抽出および塩析

通知 I 法[1]に従い、試料 5.00 g に水 20 mL を加えて 30 分間膨潤した後、アセトニトリルを用いてホモジナイズ抽出した。通知 I 法では、得られたアセトニトリル抽出液 20 mL（試料 1 g 相当）にリン酸緩衝液 20 mL および塩化ナトリウム 10 g を加えて塩析する方法を採用しているが、茶のように夾雑成分が非常に多い食品ではエマルジョンが生成することが多い。このため既報[4]においては、塩析後に遠心分離を行う方法とした。本法では、より精製効果を高めるため、塩析に供する抽出液量を減らすこととした。すなわち、通知 I 法の 1/4 量となる試料 0.25 g 相当の抽出液（5 mL）にアセトニトリル 15 mL を加えて希釈し、リン酸緩衝液 20 mL お

よび塩化ナトリウム 10 g を加えて振とう後、遠心分離（毎分3,000 回転、5 分間）を行うこととした。その結果、エマルジョンの生成しやすい烏龍茶や紅茶においても水層とアセトニトリル層の分離が改善され、高極性の夾雑成分を効果的に除去することができた。なお、最終試験溶液の濃度は、通知Ⅰ法と同様に試料 0.25 g 相当 /mL とした。塩析における各農薬の回収率を求めたところ、$logP_{ow}$ の低いニテンピラム -0.66（25℃）[5] およびメタミドホス -0.8（20℃）[6] を除き、80%以上の良好な回収率が得られた。

2) カラム精製

(1) ODS ミニカラムによる精製

通知Ⅰ法[1] では「穀類、豆類および種実類の場合」のみ、ODS ミニカラムによる精製を行うこととなっている。しかし、ODS ミニカラム精製は茶においても、緑色色素等の低極性夾雑成分の除去に有効であることから、[4] 本法では塩析後、得られたアセトニトリル層を ODS ミニカラムで精製することとした。溶出溶媒は、検討したすべての農薬が溶出するアセトニトリル 5 mL とした。

(2) グラファイトカーボン /PSA 積層ミニカラムによる精製

通知Ⅰ法ではグラファイトカーボン /NH_2（アミノプロピルシリル化シリカゲル）積層ミニカラム（500 mg/500 mg）による精製を採用しているが、グラファイトカーボン /PSA 積層ミニカラム（500 mg/500 mg）の方が色素等の夾雑成分の除去効果が高い[4] ことから、本法ではグラファイトカーボン /PSA 積層ミニカラムによる精製を検討した。

通知Ⅰ法では、試料 1 g 相当を精製に供する方法であるため、グラファイトカーボン /NH_2 積層ミニカラム精製において過負荷となり、色素等の夾雑成分がカラムから大量に溶出してしまう。これを改善するため、我々は既報[4] において、充てん量の異なるミニカラムや連結カラムを種々検討し、グラファイトカーボン /PSA 積層ミニカラム（500 mg/500 mg）とグラファイトカーボンミニカラム（500 mg）の連結カラムによる精製で、煎茶の色素を除去可能であることを示した。[4] 一方、本法では、1/4 量となる試料 0.25 g 相当を精製に用いたため、煎茶、烏龍茶、紅茶および抹茶のいずれもグラファイトカーボン /PSA 積層ミニカラム（500 mg/500 mg）のみで、十分色素を除去することができた。

試料 1 g 相当を精製に用いた場合、グラファイトカーボン /PSA 積層ミニカラムの負荷溶媒であるアセトニトリル / トルエン（3 : 1）2 mL を ODS ミニカラム精製後の残留物に加えると、油状となり、濃縮容器表面に強く付着してしまう。これは残留物にタンニン等の高極性夾雑成分が非常に多く含まれており、アセトニトリルとトルエンの混合溶媒には溶解しにくいためと考えられる。そのため既報[4] では、まずアセトニトリル 3 mL を加えて溶解後、比較的極性の低いトルエン 1 mL を加えたものをカラムへ負荷する方法とした。これに対し、本法では試料 0.25 g 相当を精製に用いたため、超音波処理を行うことにより、いずれの試料もアセトニトリル / トルエン（3 : 1）2 mL で ODS ミニカラム精製後の残留物をグラファイトカーボン /PSA 積層ミニカラムへ負荷することができた。なお、超音波処理は、煎茶や抹茶では必ずしも行う必要はないが、（半）発酵茶である烏龍茶や紅茶においては残留物がアセトニトリル / トルエン（3 : 1）に溶解しにくいため、超音波処理を行う必要があると考えられた。

グラファイトカーボン /PSA 積層ミニカラムからの各農薬の溶出挙動を検討したところ、クロルフルアズロンおよびピリミジフェンを除き、検討した農薬は 22 ～ 32 mL の画分には溶出しなかったことから、通知一斉試験法と同様に 22 mL で溶出（うち 2 mL で負荷）することとした。本条件でアシベンゾラル -*S*- メチル、アザメチホス、イソキサフルトール、オキシカルボキシン、ピラゾリネートおよびピリミジフェンを除き、70%以上の回収率が得られた。

2. 添加回収試験

市販の煎茶、烏龍茶、紅茶および抹茶を用いて、135 化合物について基準値濃度（基準値が設定されていない農薬は 0.01 ppm）で 5 併行の添加回収試験を行った。高濃度での添加回収試験では、試料 0.25 g 相当 /mL の試験溶液を LC-MS/MS に注入すると検出器においてイオンが飽和すると推測されたこと、また、添加濃度が大幅に異なる農薬の添加回収試験を同時に行うと農薬同士の干渉等の問題が発生することが予想されたことから、基準値が 1 ppm 以下の農薬と 1 ppm を超える農薬の 2 つのグループに分け、それぞれ添加回収試験を行うこととした。基準値が 1 ppm を超える農薬の添加回収試験においては、試験溶液（試料 0.25 g 相当 / mL）を 50 倍希釈（試料 0.005 g 相当 /mL）して LC-MS/MS 測定を行った。

その結果、真度の目標値（70 ～ 120%）および併行精度（RSD）の目標値（0.001 ppm ＜添加濃度≦ 0.01 ppm：25% 未満、0.01 ppm ＜添加濃度≦ 0.1 ppm：15%未満、添加濃度＞ 0.1 ppm：10%未満）[7] を満たした農薬は、検討した 135 化合物中煎茶 125 化合物、烏龍茶 94 化合物、紅茶 123 化合物、抹茶 121 化合物となり、煎茶、紅茶および抹茶では検討農薬の約 9 割で良好な結果が得られたものの、烏龍茶においては真度がやや低い農薬が多かった（Table 2）。目標値を満たさなかった農薬のうち、アザメチホス、イソキサフルトール、オキシカルボキシンおよびピラゾリネートはグラファイトカーボン /PSA 積層ミニカラム精製、メタミドホスおよびニテンピラムは塩析での損失が、真度が低い主な原因と考えられた。アシベンゾラル -*S*- メチルおよびピリミジフェンは、グラファイトカーボン /PSA 積層ミニカラムからの回収率がそれぞれ 69%および 45%と低かったが、煎茶、紅茶および抹茶の添加回収試験においては良好な真度が得られた。マトリックス共存下でのグラファイトカーボン /PSA 積層ミニカラムからの溶出挙動を検討したところ、溶出溶媒量 22 mL（うち 2 mL で負荷）でアシベンゾラル -*S*- メチルは 97%、ピリミジフェンは 90%と良好な回収率が得られたことから、両農薬はマトリックス共存下においてグラファイトカーボン /PSA 積層ミニカラムから溶出されやすくなるものと考えられた。

マトリックスの測定への影響を評価するため、溶媒標準溶液のピーク面積に対するマトリックス標準溶液のピーク面積の

Table 2. Recoveries of pesticides from fortified green tea, oolong tea, black tea, and matcha spiked at MRLs

	MRL (ppm)	Group[a)]	Green tea		Oolong tea		Black tea		Matcha	
			Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Acetamiprid	30	B	103	6	93	4	93	2	97	3
Acibenzolar-*S*-methyl	0.01	A	90	9	70	12	85	16	76	16
Acrinathrin	10	B	97	2	102	5	87	3	94	4
Aldicarb	0.05	A	91	3	70	2	86	6	87	2
Aldoxycarb	0.01	A	84	9	68	11	85	6	87	11
Anilofos	0.01	A	95	10	81	7	96	7	83	5
Aramite	0.1	A	95	5	62	3	84	2	74	2
Atrazine	0.1	A	88	5	74	4	87	3	93	2
Avermectin B1a	0.02	A	98	12	70	16	86	7	84	10
Azamethiphos	0.01	A	40	41	41	11	18	45	32	116
Azinphos methyl	0.01	A	83	10	79	11	92	18	80	14
Azoxystrobin	10	B	96	8	92	5	96	3	97	3
Bendiocarb	0.01	A	88	6	75	3	90	3	93	6
Benzofenap	0.01	A	113	6	71	11	82	15	86	6
Boscalid	0.01	A	88	9	61	10	77	13	71	12
Buprofezin	20	B	98	4	90	3	91	4	97	4
Butafenacil	0.01	A	85	7	71	5	82	9	87	8
Carbaryl	1	A	90	4	71	2	86	4	78	5
Carbofuran	0.2	A	96	5	76	1	91	4	87	2
Carpropamid	0.01	A	79	10	69	15	86	11	75	7
Chlorfluazuron	10	B	95	6	74	6	84	4	92	3
Chloridazon	0.01	A	88	7	41	51	81	5	69	14
Chloroxuron	0.1	A	91	11	74	5	86	6	88	2
Chlorpyrifos	10	B	106	7	96	5	92	2	96	3
Chlorpyrifos methyl	0.1	A	89	7	90	5	102	3	92	4
Chromafenozide	20	B	96	7	95	2	96	5	99	2
Clofentezine	20	B	39	10	70	7	90	5	81	9
Clomeprop	0.01	A	79	15	51	10	73	6	71	16
Cloquintocet mexyl	0.01	A	97	7	81	3	86	8	90	3
Clothianidin	50	B	95	7	91	4	94	5	94	2
Cumyluron	0.01	A	88	5	88	8	102	8	102	4
Cyazofamid	0.01	A	84	9	66	3	76	9	84	13
Cycloprothrin	0.5	A	67	14	38	10	51	9	52	7
Cyflufenamid	0.01	A	84	16	69	4	79	9	92	11
Cyprodinil	0.01	A	85	15	80	3	103	10	96	20
Daimuron	0.01	A	102	10	97	4	99	9	91	8
Di-allate	0.1	A	82	14	86	17	92	7	119	15
Diazinon	0.1	A	91	5	96	2	97	4	97	1
Difenoconazol	10	B	103	7	88	2	90	3	94	4
Diflubenzuron	20	B	93	6	94	4	93	8	98	2
Dimethirimol	0.01	A	84	11	60	6	47	10	81	14
Dimethomorph	0.01	A	90	12	77	4	91	10	100	6
Diuron	1	A	88	5	59	3	80	5	80	2
Epoxiconazole	0.01	A	95	11	58	10	72	9	74	6
Ethion	0.3	A	85	3	72	2	87	1	88	2
Ethiprole	10	B	106	8	96	4	89	4	93	4
Ethofenprox	10	B	97	7	88	1	89	4	94	3
Etoxazole	10	B	95	6	92	3	91	1	94	2
Fenamidone	0.01	A	89	15	71	15	78	12	76	6
Fenbuconazole	10	B	92	7	93	3	93	4	97	2
Fenobucarb	0.5	A	96	5	83	2	87	4	85	2
Fenoxaprop ethyl	0.01	A	78	14	60	16	93	4	85	8
Fenoxycarb	0.05	A	95	8	74	5	84	4	79	6
Fenpropathrin	25	B	100	4	93	3	93	3	95	2
Fenpyroximate (*E*)	10	B	95	9	90	2	91	6	96	2
Fenpyroximate (*Z*)	10	B	91	6	94	2	91	6	97	2
Ferimzone	0.01	A	89	15	72	7	81	1	91	2
Flufenacet	0.01	A	82	13	67	7	84	3	83	7
Flufenoxuron	15	B	92	8	92	3	92	4	97	1
Fluridone	0.01	A	90	11	94	3	100	9	92	7
Furametpyr	0.1	A	94	1	72	5	87	4	93	2
Furathiocarb	0.1	A	73	3	74	8	96	9	72	7
Halfenprox	10	B	97	7	91	2	89	4	92	2
Hexaflumuron	15	B	92	8	98	4	95	7	100	7
Hexythiazox	35	B	92	9	92	2	91	8	97	2
Imazalil	0.1	A	86	6	81	3	83	7	87	6
Imibenconazole	15	B	102	9	87	4	92	2	96	3
Imidacloprid	10	B	92	9	93	3	95	2	97	2

a) A: 0.25 g sample/mL, B: 0.005 g sample/mL.

Table 2. Recoveries of pesticides from fortified green tea, oolong tea, black tea, and matcha spiked at MRLs (continued)

	MRL (ppm)	Group[a)]	Green tea		Oolong tea		Black tea		Matcha	
			Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Indanofan	0.01	A	88	4	66	12	82	17	82	20
Indoxacarb	0.01	A	98	4	57	8	84	13	89	19
Iprovalicarb	0.01	A	93	13	82	3	90	9	95	12
Isoxaflutole	0.01	A	47	18	36	14	39	15	38	16
Isoxathion	5	B	100	2	95	4	94	2	94	5
Kresoxim methyl	20	B	102	5	97	2	94	2	98	3
Lactofen	0.01	A	93	6	65	6	72	8	75	10
Linuron	0.02	A	87	10	63	7	84	10	83	8
Lufenuron	10	B	83	5	99	5	83	8	98	7
Malathion	0.5	A	88	4	83	2	88	2	91	2
Mepanipyrim	0.01	A	97	4	110	13	95	20	90	12
Methabenzthiazuron	0.01	A	87	10	63	6	78	11	77	8
Methamidophos	5	B	50	10	50	2	47	3	46	5
Methidathion	1	A	91	5	83	1	90	2	93	2
Methiocarb	0.01	A	94	12	91	15	83	11	87	16
Methomyl	20	B	62	6	61	3	62	4	59	4
Methoxyfenozide	20	B	98	6	95	4	94	6	96	1
Monocrotophos	0.1	A	62	3	57	3	65	3	57	0
Monolinuron	0.05	A	91	6	86	5	88	8	79	5
Myclobutanil	20	B	102	5	93	2	95	3	98	3
Naproanilide	0.01	A	87	11	69	10	87	6	68	8
Nitenpyram	10	B	75	6	53	7	57	2	66	2
Novaluron	0.01	A	84	14	54	10	74	3	68	7
Oxamyl	0.01	A	77	13	61	11	86	9	81	16
Oxaziclomefone	0.01	A	95	10	75	11	87	4	88	11
Oxycarboxine	0.01	A	50	7	21	9	38	4	43	21
Pencycuron	0.01	A	84	4	78	5	90	8	86	7
Pentoxazone	0.01	A	94	7	46	12	75	12	62	19
Phenmedipham	0.01	A	83	9	63	6	76	17	85	18
Phosalone	2	B	102	6	97	7	92	4	95	4
Pirimicarb	0.01	A	91	4	88	4	86	7	100	8
Prochloraz	0.1	A	84	8	68	1	86	4	86	3
Profenofos	1	A	83	6	66	2	79	4	79	1
Propaquizafop	0.01	A	92	5	62	9	80	9	82	5
Propargite	5	B	102	6	96	3	92	4	98	4
Propiconazole	0.1	A	80	10	60	4	80	4	80	2
Propoxur	0.1	A	91	6	81	2	89	4	94	2
Pyraclostrobin	0.01	A	92	9	86	2	94	5	86	9
Pyrazolynate	0.02	A	30	25	19	20	13	70	21	31
Pyrazophos	0.1	A	95	7	72	2	87	6	91	4
Pyridaben	10	B	101	8	87	2	89	5	94	2
Pyriftalid	0.01	A	84	14	92	6	91	11	96	2
Pyrimidifen	5	B	84	7	63	5	71	7	89	2
Pyriproxyfen	15	B	103	6	95	2	95	3	96	4
Quinalphos	0.1	A	92	4	78	2	90	4	93	1
Quizalofop ethyl	0.01	A	88	10	57	7	77	8	88	9
Simeconazole	10	B	97	8	94	1	95	3	97	2
Spinosyn A	2	B	90	9	84	2	83	1	88	2
Spinosyn D	2	B	88	8	82	3	84	2	88	4
Spiromesifen	30	B	90	7	82	2	81	4	86	3
Tebuconazole	25	B	104	9	93	3	96	2	98	4
Tebufenozide	25	B	97	9	98	4	94	7	97	4
Tebuthiuron	0.02	A	93	6	72	5	91	9	86	7
Teflubenzuron	20	B	87	7	87	5	93	9	97	4
Tetrachlorvinphos	0.01	A	86	13	73	8	79	9	81	8
Tetraconazole	20	B	104	7	96	3	93	3	96	4
Thiabendazole	0.1	A	19	52	3	34	0	-	3	167
Thiacloprid	30	B	96	7	94	3	94	4	97	1
Thiamethoxam	15	B	89	9	88	3	89	2	88	1
Triadimefon	1	A	89	6	76	2	87	5	92	2
Triadimenol	20	B	103	7	91	4	94	2	97	3
Tridemorph	20	B	80	8	72	2	65	7	71	2
Trifloxystrobin	5	B	101	6	97	4	91	2	95	2
Triflumizole	15	B	89	8	108	6	80	6	71	2
Triflumuron	0.02	A	95	8	54	10	71	10	74	9
Triticonazole	0.01	A	80	15	51	9	82	3	96	5
XMC	10	B	101	4	94	4	93	2	96	2

[a)] A: 0.25 g sample/mL, B: 0.005 g sample/mL.

比を求めた。烏龍茶では、その他の試料と比較して 0.80 未満となった農薬が多かったことから、夾雑成分によるイオン化阻害が烏龍茶において真度が低い農薬が多い原因と推察された。煎茶、烏龍茶、紅茶および抹茶のいずれの試料においても 0.80 未満となった農薬は、アザメチホス、シクロプロトリン、メソミルおよびモノクロトホスであった。Fig. 2 に煎茶のブランク試験溶液の TIC（total ion current）クロマトグラムを示した。いずれの試料においてもメソミルおよびモノクロトホスの保持時間（8.3 分）付近にカフェイン由来の大きなピークが見られたことから、茶に大量に含まれるカフェインによるイオン化阻害が、メソミルおよびモノクロトホスの真度が低い原因と推察された。マトリックスによる影響が大きかった農薬のうち、感度が十分得られるものについて試験溶液を希釈して測定を行った結果、煎茶 3 化合物、烏龍茶 24 化合物、紅茶 2 化合物、抹茶 2 化合物で真度および併行精度の目標値を満たした（Table 3）。なお、これらの農薬は、試験溶液を希釈した場合においても S/N ≧ 10 が得られた。茶は夾雑成分が非常に多く、測定においてマトリックスの影響を受けやすいことから、感度が十分得られる場合は試験溶液を希釈して測定するのがよいと考えられた。

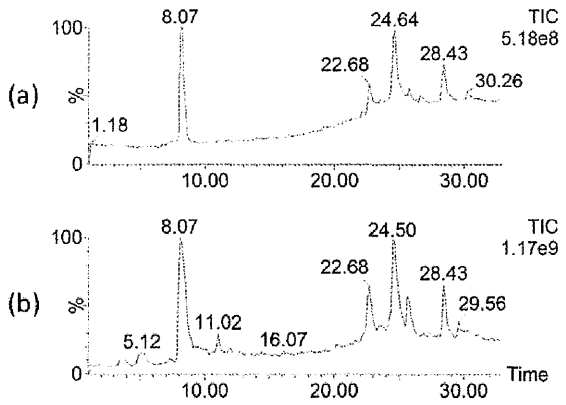

Fig. 2. TIC (total ion current) chromatograms of blank extracts of green tea
(a) 0.005 g sample/mL, (b) 0.25 g sample/mL.
Scan range *m/z* 100–1000; ESI (+); cone voltage 50 V.

検量線は、いずれの農薬も良好な直線性（r^2 ≧ 0.99）が得られた。ブランク試験溶液を測定した結果、煎茶、烏龍茶、紅茶および抹茶のいずれの試料においても、検討したすべての農薬で定量を妨害するピークの面積は添加試料から得られるピークの面積の 1/10 未満であり、選択性に問題はなかった。なお、基準値が 0.01 ppm を超える農薬については、0.01 ppm での添加回収試験は行っていないが、試料中濃度 0.01 ppm 相当のマトリックス標準溶液を用いて S/N 比を求めたところ、いずれも S/N ≧ 10 が得られた。

Table 3. Recoveries of pesticides from fortified tea after dilution of the test solutions with methanol

	Sample	MRL (ppm)	Group [a]	Dilution factor	Recovery	
					Mean (%)	RSD (%)
Clofentezine	Green tea	20	B	10	71	12
Methomyl	Green tea	20	B	10	84	8
Monocrotophos	Green tea	0.1	A	10	70	5
Aldoxycarb	Oolong tea	0.01	A	2.5	75	8
Aramite	Oolong tea	0.1	A	10	100	15
Boscalid	Oolong tea	0.01	A	2.5	74	7
Carpropamid	Oolong tea	0.01	A	2.5	100	7
Clomeprop	Oolong tea	0.01	A	2.5	70	11
Cyazofamid	Oolong tea	0.01	A	2.5	90	24
Cyflufenamid	Oolong tea	0.01	A	2.5	81	4
Diuron	Oolong tea	1	A	10	93	9
Epoxiconazole	Oolong tea	0.01	A	2.5	71	15
Fenoxaprop ethyl	Oolong tea	0.01	A	2.5	86	10
Flufenacet	Oolong tea	0.01	A	2.5	87	11
Linuron	Oolong tea	0.02	A	2.5	92	10
Methabenzthiazuron	Oolong tea	0.01	A	2.5	75	8
Methomyl	Oolong tea	20	B	10	74	9
Monocrotophos	Oolong tea	0.1	A	10	96	6
Naproanilide	Oolong tea	0.01	A	2.5	86	22
Oxamyl	Oolong tea	0.01	A	2.5	89	9
Phenmedipham	Oolong tea	0.01	A	2.5	80	22
Prochloraz	Oolong tea	0.1	A	10	98	19
Profenofos	Oolong tea	1	A	10	100	18
Propaquizafop	Oolong tea	0.01	A	2.5	76	12
Propiconazole	Oolong tea	0.1	A	10	108	14
Quizalofop ethyl	Oolong tea	0.01	A	2.5	71	9
Triticonazole	Oolong tea	0.01	A	2.5	70	12
Methomyl	Black tea	20	B	10	73	9
Monocrotophos	Black tea	0.1	A	10	92	12
Methomyl	Matcha	20	B	10	75	4
Monocrotophos	Matcha	0.1	A	10	78	9

[a] A: 0.25 g sample/mL, B: 0.005 g sample/mL.

Ⅳ 結論

通知Ⅰ法（「LC-MS（/MS）による農薬等の一斉試験法Ⅰ（農産物）」）を改良し、茶を対象とした一斉分析法を開発した。主な改良点は、①塩析およびミニカラム精製に供する試料量を1/4量（試料0.25 g相当）に変更したこと、②ODSミニカラム精製を追加したこと、および③グラファイトカーボン/NH_2積層ミニカラム精製をグラファイトカーボン/PSA積層ミニカラム精製に変更したことである。本法は、通知Ⅰ法と比較して夾雑成分の除去効果が大幅に改善した。確立した方法で、煎茶、烏龍茶、紅茶および抹茶を用いて基準値濃度（基準値が設定されていない農薬は0.01 ppm）で5併行の添加回収試験を行った結果（Table 3に示した農薬/試料の組み合わせについてはマトリックスの影響を軽減するため希釈して測定）、検討した135化合物中煎茶128化合物、烏龍茶118化合物、紅茶125化合物、抹茶123化合物が真度および併行精度の目標値を満たした。検討したすべての農薬において選択性に問題はなかった。これらの結果から、本法は茶の規格基準への適否判定のための分析法として適用可能であると考えられた。

本研究は「厚生労働省医薬食品局食品安全部残留農薬等に関するポジティブリスト制度導入に係る分析法開発事業」により実施した。

Ⅴ 文献

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Original

Multiresidue Analysis of Pesticides in Vegetables and Fruits by Supercritical Fluid Extraction and Liquid Chromatography-Tandem Mass Spectrometry

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A multiresidue method for analyzing pesticides in vegetables and fruits by supercritical fluid extraction (SFE) and LC-MS/MS was developed. The sample preparation and SFE parameters were optimized for extracting LC-amenable polar and medium-polarity pesticides. High recoveries were achieved for most of the tested pesticides by extracting a 1 : 1 : 1 sample–Celite–anhydrous magnesium sulfate mixture with supercritical carbon dioxide at 16.4 MPa at 40℃ for 30 min with methanol added as a modifier. The recoveries of 117 pesticides fortified with 0.01 mg/kg of each pesticide were 70–120%, and the relative standard deviations were less than 25% for 112 pesticides in tomato and 103 pesticides in cucumber. No significant differences were observed in the residue concentrations determined in real samples by the SFE method and the liquid extraction method (the modified Japanese official method). Higher recoveries of polar pesticides, such as acephate and methamidophos, were achieved by the developed SFE method than by the liquid extraction method.

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Key words: pesticides; supercritical fluid extraction; LC-MS/MS

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Introduction

Supercritical fluids possess liquid-like solvating properties but gas-like diffusion properties and viscosities that enable them to rapidly penetrate substrates, and solutes can often be extracted faster by a supercritical fluid than by a liquid[1]. A number of supercritical fluid extraction (SFE) methods have been developed as alternatives to conventional liquid extraction methods for determining pesticide residues in foods, such as vegetables and fruits[2)–10)], cereals[11)–16)], eggs[17), 18)], meat[19)], oil[20)], baby food[21)], and honey[22)]. The main advantages of SFE are that it can lead to less solvent use and that it can be automated, reducing labor costs. In addition, adjusting the solvating power of the supercritical fluid by changing the pressure and temperature can permit selective extraction of analytes. Supercritical carbon dioxide (CO_2) is commonly used in SFE because of its low critical point (critical temperature 31.2℃, critical pressure 72.8 atm), its lack of toxicity and flammability, and its availability at high purity and low cost. Supercritical CO_2 can also be easily removed from a sample by decreasing the pressure after extraction. However, the low polarity of CO_2 limits the range of extractable pesticides; thus, most of the reported SFE methods have been applied for relatively low-polarity pesticides that can be analyzed by gas chromatography (GC). The addition of polar solvents (*e.g.*, methanol or water) as modifiers to the sample matrix or fluids increases the polarity of supercritical CO_2, improving the extraction efficiency of relatively polar pesticides[1), 23), 24)]. However, up to now, only a few multiresidue methods have been published for liquid chromatography (LC)-amenable polar and medium-polarity pesticides by employing a combination of SFE and LC-photodiode array (PDA) or LC-mass spectrometry (MS or MS/MS) [3)].

Although the addition of water to supercritical CO_2 can improve the extraction efficiency of relatively polar pesticides, excessive water in the samples must be removed or controlled before SFE, because water can block the restrictor during extraction[23)]. Polar pesticides also preferentially partition into the aqueous phase (rather than supercritical CO_2), resulting in poor recoveries if a significant amount of water is present. The simplest ways of removing water from a sample are to air-dry, oven-dry, or freeze-dry the sample before extraction.

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However, these methods are not ideal because they are time-consuming and can result in loss of volatile and semi-volatile pesticides[25]. Instead of removing water from the sample, drying agents, such as diatomaceous earth (Hydromatrix, Celite No. 545), polymers, cellulose, magnesium sulfate, or sodium sulfate have been used to control the moisture content[4),25)-29)]. Diatomaceous earth and polymers are efficient absorbents, but their use results in poor recoveries of polar pesticides[4),23)]. Valverde-Garcia *et al.* achieved high recoveries of polar methamidophos and other GC-amenable pesticides by using anhydrous magnesium sulfate as a drying agent[28)], as did Eller *et al.* with a sample–Hydromatrix–magnesium sulfate (2 : 1 : 2) mixture[29)].

We aimed to examine the applicability of SFE for determining LC-amenable polar and medium-polarity pesticides and to develop a reliable and sensitive multiresidue method for the analysis of these pesticides in vegetables and fruits by means of SFE and LC-MS/MS. The sample preparation and SFE parameters were optimized for the simultaneous extraction of polar and medium-polarity pesticides from vegetables and fruits. The recoveries from fortified samples using the proposed SFE method were compared with those obtained from a conventional liquid extraction method. In addition, concentrations of incurred residues in real samples were also analyzed by the SFE method and compared with those determined by the liquid extraction method.

Materials and Methods

1. Reagents and materials

1.1 Solvents and chemicals

Pesticide-analysis-grade acetone, acetonitrile, methanol, and toluene were purchased from Kanto Chemical (Tokyo, Japan). Pesticide-analysis-grade sodium chloride and analytical-grade ammonium sulfate, anhydrous magnesium sulfate, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). LC-MS-grade methanol and water (Kanto Chemical) were used for LC-MS/MS analyses. Diatomaceous earth (Celite, No. 545) was purchased from Wako Pure Chemical Industries, Ltd., and glass microfiber filters (GF/F) from Whatman (Maidstone, UK). The water used to prepare the test solutions was purified in an NZJ-2DSYW distillation apparatus (Fujiwara Scientific, Tokyo, Japan).

Phosphate buffer (0.5 mol/L, pH 7.0) was prepared by dissolving 52.7 g dipotassium hydrogen phosphate and 30.2 g potassium dihydrogen phosphate in 500 mL of water. The pH of the solution was adjusted to 7.0 by adding 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid and adjusting the final volume to 1 L with water.

1.2 Analytical standards

Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Hayashi Pure Chemical (Osaka, Japan), Wako Pure Chemical, and Kanto Chemical. Individual stock standard solutions (1,000 mg/L) were prepared in acetonitrile or methanol, depending on the solubility of the pesticide in each solvent. Working solutions were prepared by mixing the stock standard solutions and diluting with methanol. Calibration standard solutions were freshly prepared by diluting the working standard solutions with methanol.

1.3 Cartridge columns

Octadecylsilyl silica gel (ODS) columns (Mega Bond Elut C18, 1,000 mg) were obtained from Agilent Technologies (Palo Alto, CA, USA), and tandem graphitized carbon/primary secondary amine (PSA) columns (InertSep GC/PSA, 500 mg/500 mg) were obtained from GL Sciences (Tokyo, Japan).

2. Apparatus

2.1 SFE

The supercritical fluid extractor (Jasco, Tokyo, Japan) consisted of a liquid CO_2 pump (SCF-Get), a solvent pump (PU-2080), an oven with a six-vessel changer (SCF-Evc-Sr), a back pressure regulator (SCF-Bpg), a multi-channel detector (MD-2010), a stainless steel extraction vessel (10 mL, 127 mm length, 10 mm i.d.), and an ODS trap column (50 mm length, 4.6 mm i.d., 30 μm particle size). The optimal SFE conditions were as follows: extraction pressure, 16.4 MPa; extraction temperature, 40℃; CO_2 flow rate, 2.0 mL/min; extraction time, 30 min; ODS trap temperature, 40℃. After extraction, the extracts were eluted from the ODS trap column with 2 mL of acetonitrile at a flow rate of 2 mL/min. The column was then washed with 10 mL of acetone–hexane (1 : 1) and preconditioned with 10 mL of acetonitrile before the next extraction.

2.2 LC-MS/MS

An Alliance 2695 LC system (Waters, Milford, MA, USA) coupled to a Quattro Premier mass spectrometer (Waters) was used, with the following operating conditions: Inertsil ODS-4 column (150 mm length, 2.1 mm i.d., 3 μm particle size; GL Sciences) with an Inertsil ODS-4 guard column (10 mm length, 1.5 mm i.d., 3 μm particle size; GL Sciences); mobile phases, 5 mmol/L ammonium acetate in water (Solvent A) and 5 mmol/L ammonium acetate in methanol (Solvent B); solvent gradient, 15% Solvent B at 0 min, 40% Solvent B at 1 min, 40% Solvent B at 3.5 min, 50% Solvent B at 6 min, 55% Solvent B at 8 min, 95% Solvent B at 17.5 min, 95% Solvent B at 33 min, 100% Solvent B at 33.1 min, 100% Solvent B at 43 min, and 15% Solvent B at 43.1 min; flow rate, 0.2 mL/min; column temperature, 40℃; injection volume, 5 μL; ionization mode, electrospray ionization (ESI) positive mode, capillary voltage, 3 kV; source temperature, 120℃; desolvation temperature, 400℃; desolvation gas, nitrogen at 800 L/hr; cone gas, nitrogen at 50 L/hr; collision gas, argon at 3.1×10^{-3} mbar. The selective reaction monitoring (SRM) transitions and retention times are summarized in Supplemental Table S1.

2.3 Laboratory apparatus

A homogenizer (Polytron PT 10–35 GT; Kinematica, Lucerne, Switzerland), food processor (Grindomix GM200; Retsch, Haan, Germany), rotary evaporator (N-1000/NVC-2100; Tokyo Rikakikai, Tokyo, Japan), electric

shaker (SR-2w; Taitec, Saitama, Japan), and vacuum pump (APN-215MV-1; Iwaki, Tokyo, Japan) connected to a Kiriyama funnel containing filter paper No. 5B (Kiriyama Glass Works, Tokyo, Japan) were used.

3. Sample preparation

3.1 SFE

Five grams of Celite was added to 5.0 g of sample, and after mixing, 5.0 g of anhydrous magnesium sulfate was added. The sample was thoroughly mixed using a mortar and pestle, then transferred to a 10 mL extraction vessel, and 0.2 mL of methanol was added to the SFE sample from the bottom of the vessel as a modifier. A glass microfiber filter was cut into disks, which were placed at each end of the vessel, to prevent particles leaving the vessel and affecting the SFE system.

The extract was eluted from the ODS trap column with 2 mL of acetonitrile, concentrated to approximately 0.5 mL using a rotary evaporator (below 40℃), and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 3 mL of methanol.

3.2 Liquid extraction

Test solutions were prepared according to the Japanese official method "Multiresidue Method I for Agricultural Chemicals by LC/MS (Agricultural Products)", except for some modifications to the cleanup column used and the final volume. Twenty grams of the sample was weighed in a 250 mL glass tube and homogenized with 50 mL of acetonitrile. The homogenate was filtered under vacuum, and the residue was re-homogenized with 20 mL of acetonitrile and then filtered again. The extracts were combined, and the volume was made up to 100 mL with acetonitrile.

A 20 mL aliquot of the extract was added to a 100 mL separatory funnel containing 10 g of sodium chloride and 20 mL of phosphate buffer (0.5 mol/L, pH 7.0) and shaken vigorously for 10 min. The acetonitrile layer was loaded onto an ODS column that had been preconditioned with 10 mL of acetonitrile and then eluted with 5 mL of acetonitrile. The combined eluate was concentrated to approximately 1 mL using a rotary evaporator (below 40℃) and evaporated to dryness under a stream of nitrogen.

The residue was redissolved in 2 mL of acetonitrile–toluene (3 : 1), loaded onto a tandem graphitized carbon/PSA column, and eluted with 20 mL of acetonitrile-toluene (3 : 1). The combined eluate was concentrated to approximately 1 mL using a rotary evaporator (below 40℃) and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 8 mL of methanol.

Results and Discussion

1. Sample preparation optimization for SFE

Vegetable and fruit samples usually contain large amounts of water; hence, excess water must be removed or controlled before SFE to prevent water from affecting the instrument performance. Therefore, the sample preparation for SFE was optimized for the determination of LC-amenable polar and medium-polarity pesticides in vegetables and fruits. Eight pesticides, representing a wide range of polarities, were used in the optimization process. The pesticides were acephate, methamidophos, monocrotophos, carbaryl, azoxystrobin, acibenzolar-*S*-methyl, flufenoxuron, and acrinathrin, which have log P_{ow} values of −0.89, −0.8, −0.22, 1.85, 2.5, 3.1, 4, and 5.6, respectively. Tomatoes were used as model high-water-content samples. Methanol (0.2 mL) as a modifier was added to the sample before SFE. All experiments were performed in duplicate. Anhydrous magnesium sulfate has been reported to be an efficient drying agent, giving high recoveries of polar methamidophos[28]. However, adding anhydrous magnesium sulfate to a sample that contains a large amount of water will

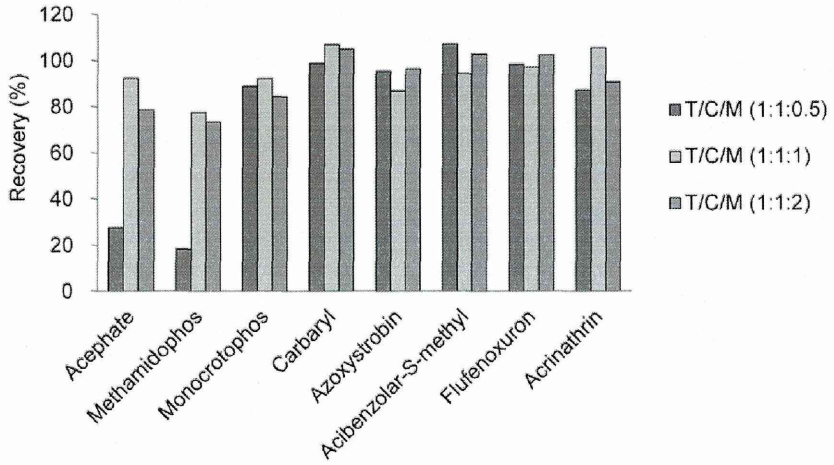

Fig. 1. Effect of adding Celite and anhydrous magnesium sulfate on the recoveries of eight pesticides from fortified tomato samples by supercritical fluid extraction (SFE)

T, tomato; C, Celite; M, anhydrous magnesium sulfate.
SFE conditions: extraction pressure, 16.4 MPa; extraction temperature, 40℃ (corresponding to a CO_2 density of 0.8 g/mL); extraction time, 30 min; modifier, methanol (0.2 mL)

cause generation of heat and formation of agglomerates. Therefore, we mixed the samples with Celite before adding anhydrous magnesium sulfate. The amounts of Celite and anhydrous magnesium sulfate were optimized by testing various proportions of sample, Celite, and anhydrous magnesium sulfate. As shown in Fig. 1, a 1 : 1 : 0.5 sample–Celite–anhydrous magnesium sulfate mixture gave poor recoveries of acephate and methamidophos. However, a 1 : 1 : 1 sample–Celite–anhydrous magnesium sulfate mixture gave high recoveries for the polar pesticides and the relatively low-polarity pesticides, such as acrinathrin. Mixing the sample with both Celite and anhydrous magnesium sulfate gave a powdery and dispersed sample that was suitable for SFE. A 1 : 1 : 2 sample–Celite–anhydrous magnesium sulfate mixture also gave high recoveries for all of the tested pesticides, but using more of the drying agents led to lower loading of the sample in the extraction vessel. Replacing anhydrous magnesium sulfate with anhydrous sodium sulfate also gave a good recovery for monocrotophos (92%), but it gave poor recoveries for acephate (34%) and methamidophos (23%). We concluded that a 1 : 1 : 1 sample–Celite–anhydrous magnesium sulfate mixture was the optimum sample preparation for SFE.

2. Optimization of SFE conditions

The solvating power of a supercritical fluid is highly dependent on its density (which can be controlled by changing the temperature and pressure) and the presence of modifiers. Therefore, SFE parameters such as CO_2 density, extraction temperature, extraction time, and modifiers were optimized for the simultaneous extraction of polar and medium-polarity pesticides from vegetables and fruits using the same set of eight pesticides as for the sample preparation optimization. All of the experiments were performed in duplicate and involved extracting the pesticides from fortified tomato samples. Methanol (0.2 mL) was added to the SFE sample as a modifier, except for the experiments on modifier optimization. Optimal SFE conditions were obtained by sequentially varying one parameter while maintaining all of the other parameters fixed.

First, the CO_2 density was optimized by testing densities of 0.3, 0.5, 0.7, and 0.8 g/mL (corresponding to pressures of 8.2, 9.1, 11.4, and 16.4 MPa, respectively) at 40℃. The results of these tests are shown in Fig. 2. At a CO_2 density of 0.3 g/mL, the recoveries of all of the tested pesticides, especially the polar pesticides such as acephate and methamidophos, were low (<70%). Increasing the CO_2 density improved the recoveries, and the best recoveries for all of the pesticides tested were observed at a density of 0.7–0.8 g/mL. Thus, we concluded that a density of 0.8 g/mL is optimal for extraction.

The effect of the extraction temperature on the recoveries was evaluated at 32, 40, and 50℃, corresponding to pressures of 12.4, 16.4, and 21.4 MPa, respectively, at a fixed CO_2 density of 0.8 g/mL. The pesticide recoveries were not clearly influenced by temperature over the range tested with a fixed CO_2 density. However, most of the tested pesticides showed slightly higher recoveries at 40℃. Since it is better to perform extraction at low temperature, especially for thermally labile analytes, further experiments were carried out at 40℃.

The extraction time also influences the extraction efficiency; thus, the recoveries at extraction times of 10, 15, and 30 min were compared with the extraction pressure and temperature set at 16.4 MPa and 40℃, respectively (giving a CO_2 density of 0.8 g/mL). The recoveries of all of the tested pesticides were significantly improved by increasing the extraction time from 10 to 15 min. Although the recoveries of most of the pesticides were affected only minimally, the recoveries of the polar pesticides were slightly improved by prolonging the extraction time from 15 to 30 min. In particular, the recovery of methamidophos increased from 37% (10 min) to 78% (30 min). We concluded that an extraction time of 30 min was appropriate for extracting pesticides with

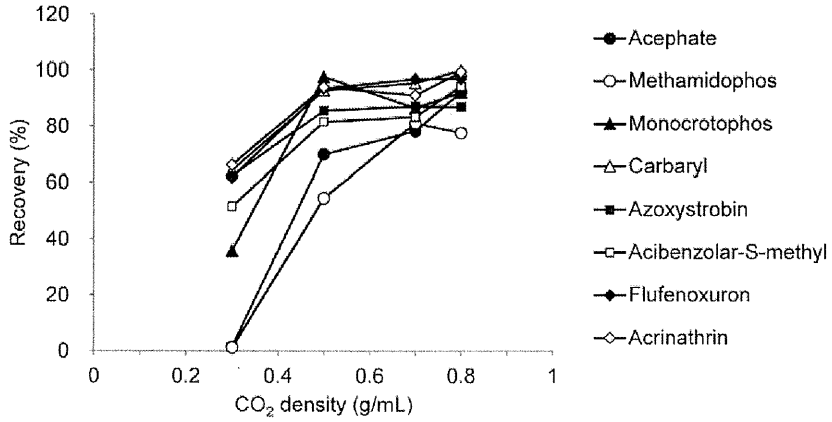

Fig. 2. Effect of CO_2 density on the recoveries of eight pesticides from fortified tomato samples by supercritical fluid extraction (SFE)

SFE conditions: extraction temperature, 40℃; extraction time, 30 min; modifier, methanol (0.2 mL); SFE sample, tomato–Celite–anhydrous magnesium sulfate (1 : 1 : 1)

a wide range of polarities.

Supercritical CO_2 has a limited ability to dissolve polar pesticides, but its solvating power can be enhanced by adding a polar solvent, which serves as a modifier, to the fluid or to the sample. Therefore, the effect of adding modifiers on the pesticide recoveries was evaluated. Three organic solvents (acetone, acetonitrile, and methanol) were tested as potential modifiers. For each of the tests, 0.2 mL of one of the solvents was added directly to an SFE sample before extraction, which is the simplest method for introducing modifiers[1]. The results of the tests are shown in Fig. 3. High recoveries were achieved for the relatively low-polarity pesticides without a modifier, and adding a modifier had only a marginal influence on the recoveries. In contrast, the polar pesticides (acephate, methamidophos, and monocrotophos) gave poor recoveries without a modifier but high recoveries when a modifier was added. Methanol was the most effective modifier, leading to high recoveries for all of the pesticides tested. The optimal amount of methanol to be added was investigated by varying the volume added within the range of 0.1–0.5 mL (Fig. 4). The recoveries of the polar pesticides were improved when 0.1–0.2 mL of methanol was added, but they decreased when more methanol was added, indicating that supercritical CO_2 was oversaturated with methanol when more than

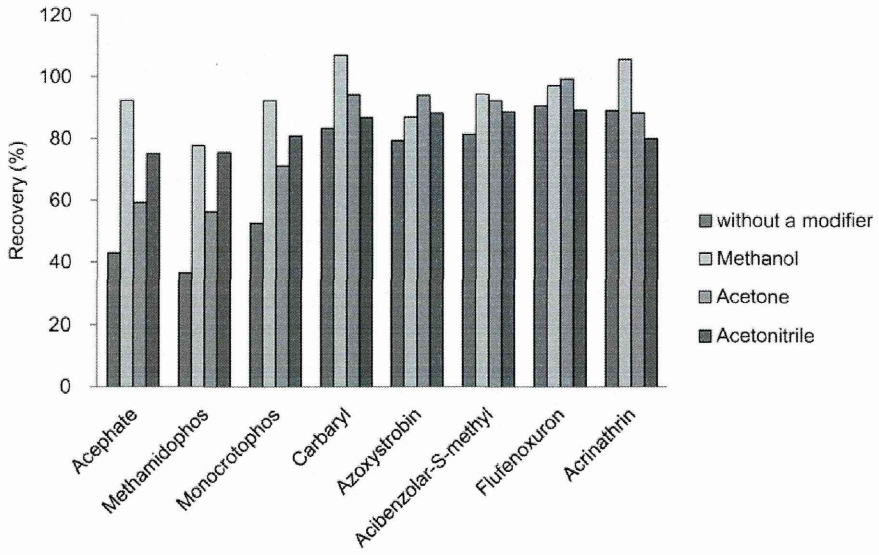

Fig. 3. Effect of adding modifiers (0.2 mL) on the recoveries of eight pesticides from fortified tomato samples by supercritical fluid extraction (SFE)

SFE conditions: extraction pressure, 16.4 MPa; extraction temperature, 40℃ (corresponding to a CO_2 density of 0.8 g/mL); extraction time, 30 min; SFE sample, tomato–Celite–anhydrous magnesium sulfate (1 : 1 : 1)

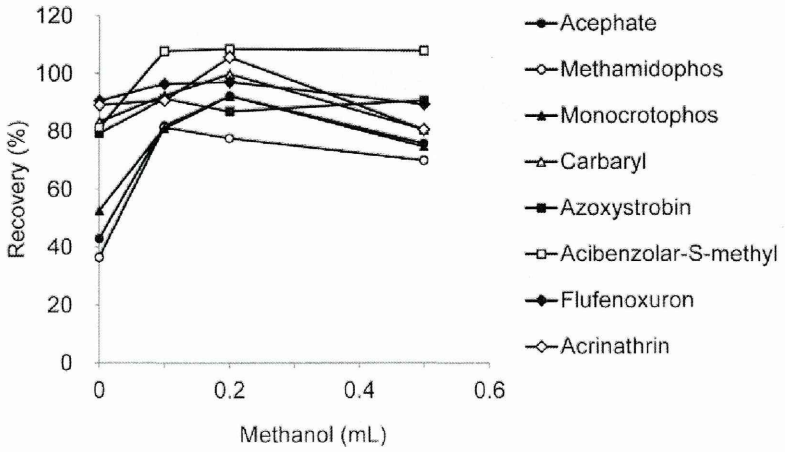

Fig. 4. Effect of adding different amounts of methanol on the recoveries of eight pesticides from fortified tomato samples by supercritical fluid extraction (SFE)

SFE conditions: extraction pressure, 16.4 MPa; extraction temperature, 40℃ (corresponding to a CO_2 density of 0.8 g/mL); extraction time, 30 min; SFE sample, tomato–Celite–anhydrous magnesium sulfate (1 : 1 : 1)

Table 1. Recoveries of pesticides from fortified tomato and cucumber samples by the supercritical fluid extraction (SFE) method and a liquid extraction method

	SFE				Liquid extraction			
	Tomato		Cucumber		Tomato		Cucumber	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Acephate	92	4	93	6	0	—	18	13
Acetamiprid	99	3	90	5	83	3	91	5
Acibenzolar-S-methyl	98	13	86	15	66	13	93	17
Acrinathrin	92	8	86	15	54	24	68	10
Aldoxycarb	105	8	92	4	82	12	72	9
Anilofos	79	9	93	4	93	6	89	7
Aramite	77	8	88	3	77	7	92	8
Atrazine	90	3	88	4	89	7	93	4
Avermectin B1a	89	11	40	54	99	10	76	16
Azinphos methyl	86	4	91	11	83	4	88	6
Azoxystrobin	90	9	90	4	83	6	91	5
Bendiocarb	82	7	87	6	89	2	90	5
Benzofenap	84	9	78	9	85	5	90	6
Boscalid	72	10	78	7	97	8	94	10
Buprofezin	83	4	81	6	73	9	90	4
Butafenacil	85	6	87	4	70	15	92	8
Carbaryl	90	12	84	6	90	7	75	9
Carbofuran	86	7	97	3	94	3	93	4
Carpropamid	83	9	84	6	88	7	86	12
Chlorfluazuron	77	4	73	4	37	9	24	12
Chloridazon	79	8	69	7	85	4	85	8
Chloroxuron	86	7	88	8	95	4	87	8
Chlorpyrifos	77	13	92	5	79	8	83	5
Chlorpyrifos methyl	70	14	98	12	93	13	86	14
Chromafenozide	90	8	90	5	83	3	92	8
Clofentezine	66	8	18	20	15	33	77	11
Clomeprop	77	10	81	10	91	9	89	8
Cloquintocet mexyl	86	6	90	4	90	5	91	6
Clothianidin	34	20	32	31	64	4	78	10
Cumyluron	91	8	87	8	107	5	94	4
Cyazofamid	84	9	87	7	96	3	89	7
Cyflufenamid	85	17	74	9	84	7	85	10
Cyprodinil	92	12	70	20	97	8	85	9
Daimuron	92	9	88	3	97	5	91	8
Diazinon	87	10	90	6	89	5	95	5
Difenoconazol	73	7	73	5	89	5	90	5
Diflubenzuron	84	10	81	4	79	9	84	8
Dimethomorph	91	4	87	5	87	4	92	5
Diuron	91	3	84	1	87	4	91	6
Epoxiconazole	80	12	77	6	91	3	81	4
Ethion	79	7	85	1	90	3	89	4
Ethofenprox	77	9	72	5	91	4	93	6
Etoxazole	73	7	69	6	89	4	92	3
Fenamidone	79	8	14	35	89	7	87	10
Fenbuconazole	71	5	72	5	85	2	89	4
Fenobucarb	94	7	92	12	88	4	87	6
Fenoxaprop ethyl	84	11	86	6	83	5	86	9
Fenoxycarb	84	6	87	4	88	5	93	2
Fenpropathrin	71	6	81	8	90	3	77	2
Fenpyroximate (*E*)	78	6	79	4	81	6	81	6
Fenpyroximate (*Z*)	78	6	79	4	84	4	86	8
Ferimzone	82	7	77	3	88	3	90	8
Flufenacet	77	7	84	12	93	2	87	7
Flufenoxuron	75	10	70	4	78	5	83	9
Fluridone	91	4	90	6	91	5	87	2
Furametpyr	98	5	65	11	88	6	84	12
Furathiocarb	81	7	69	6	89	6	87	11
Halfenprox	76	10	61	3	94	8	89	4

Table 1. Continued

	SFE				Liquid extraction			
	Tomato		Cucumber		Tomato		Cucumber	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Imidacloprid	80	11	81	10	83	6	83	5
Indoxacarb	78	13	78	9	79	12	98	13
Iprovalicarb	82	10	95	5	89	6	93	7
Isoxathion	85	6	85	8	94	5	91	1
Kresoxim methyl	83	10	88	7	92	7	95	11
Lactofen	79	13	82	4	82	6	98	4
Linuron	85	5	84	7	88	7	88	8
Malathion	92	6	83	8	86	5	90	5
Mepanipyrim	88	7	89	13	89	7	106	12
Methabenzthiazuron	90	5	90	5	93	7	89	5
Methamidophos	94	6	88	5	30	6	34	10
Methidathion	91	4	88	4	85	7	91	5
Methiocarb	86	8	80	5	88	12	101	8
Methomyl	8	64	86	23	98	6	103	6
Methoxyfenozide	84	7	89	5	75	8	91	7
Monocrotophos	100	6	89	5	74	6	76	4
Monolinuron	83	6	87	8	82	11	87	12
Myclobutanil	82	7	81	4	94	8	94	4
Naproanilide	75	9	81	9	90	4	89	8
Nitenpyram	0	—	22	75	43	12	50	10
Novaluron	74	13	64	2	91	4	90	8
Oxamyl	12	33	60	47	78	6	80	8
Oxaziclomefone	82	6	84	5	89	4	90	3
Oxycarboxine	86	3	88	4	33	14	59	11
Pencycuron	83	10	80	4	92	6	89	7
Phenmedipham	91	11	91	6	74	3	83	11
Phosalone	79	8	78	5	87	3	94	1
Pirimicarb	74	6	83	11	93	5	89	8
Prochloraz	72	6	59	7	89	4	90	7
Profenofos	89	6	90	2	85	6	96	1
Propaquizafop	80	9	80	4	89	5	86	7
Propargite	75	13	87	4	86	5	96	8
Propiconazole	80	6	81	4	91	7	90	5
Propoxur	88	2	86	4	85	5	90	4
Pyraclostrobin	88	4	89	6	91	6	87	6
Pyrazophos	83	5	89	2	89	7	94	3
Pyridaben	74	7	75	4	80	3	84	5
Pyriftalid	90	10	94	5	86	7	91	7
Pyrimidifen	87	5	81	4	32	13	40	14
Pyriproxyfen	75	9	81	3	92	5	94	4
Quinalphos	88	6	92	5	92	4	88	9
Quizalofop ethyl	83	7	77	4	92	6	88	6
Simeconazole	87	6	80	4	89	4	83	8
Spiromesifen	83	8	78	3	77	7	84	4
Tebuconazole	79	6	77	4	89	4	88	6
Tebufenozide	94	7	85	6	74	6	87	7
Tebuthiuron	94	3	91	4	87	3	91	7
Teflubenzuron	79	7	85	13	88	24	117	16
Tetrachlorvinphos	79	8	75	9	91	5	84	9
Tetraconazole	87	9	69	5	93	4	88	3
Thiacloprid	73	6	78	2	75	3	88	5
Thiamethoxam	77	8	77	8	76	3	80	5
Triadimefon	83	9	91	5	94	6	93	5
Triadimenol	83	7	82	6	84	7	91	6
Trifloxystrobin	82	9	85	4	95	5	91	3
Triflumuron	73	9	81	12	94	5	90	10
Triticonazole	78	6	70	5	90	4	82	6
XMC	90	5	86	6	90	3	95	8

0.2 mL of methanol was added. We concluded that 0.2 mL of methanol (added to the SFE sample) was an optimal modifier for extracting polar and medium-polarity pesticides.

We also optimized the elution solvent from the ODS trap column. High recoveries were achieved for all of the tested pesticides when the trap column was eluted with methanol, but co-extracted low-polarity matrix components, such as chlorophylls, were also eluted. In contrast, acetonitrile eluted relatively small amounts of matrix components from the ODS trap column, but achieved high recoveries of the tested pesticides. The acetonitrile eluate was suitable for subsequent LC-MS/MS analysis without any additional cleanup. Therefore, we chose acetonitrile to elute extracts from the ODS trap column.

3. Recovery tests

The recoveries and relative standard deviations (RSDs) obtained by the developed SFE method and the liquid extraction method from five replicates of tomato and cucumber fortified with 0.01 mg/kg of 117 pesticides are presented in Table 1. The liquid extraction method used was the Japanese official method, with modifications to the cleanup column and the final volume. The tandem graphitized carbon/aminopropylsilanized silica gel (NH_2) column was replaced with a tandem graphitized carbon/PSA column, which removes acidic co-extractives more efficiently. For the SFE method, out of 117 pesticides tested, 112 pesticides (96% of the pesticides) in tomato samples and 103 pesticides (88% of the pesticides) in cucumber samples gave recoveries of 70–120%, with RSDs of less than 25%. Poor recoveries (< 40%) were observed for neonicotinoid insecticides, nitenpyram and clothianidin, which possess a nitromethylene or a nitroimino group, respectively. This may be caused by interactions between the matrices and the nitro groups on the pesticides, but further investigations are required to clarify the mechanism. There were significant differences between the recoveries from tomato and cucumber for several pesticides (avermectin B1a, clofentezine, fenamidone, methomyl, and oxamyl). The dependency of the extraction efficiency on the matrix type is presumably caused by interactions between the analyte and matrix components[30], and further studies are needed to solve this problem. Matrix effects were evaluated by comparing analyte peak areas in matrix-matched standards and standards in solvent. The peak area ratios of matrix-matched standards to standards in solvent were in the range of 0.80–1.20, indicating that there were no significant matrix effects.

For the liquid extraction method, out of the 117 pesticides tested, 106 (91%) in the tomato samples and 110 (94%) in the cucumber samples were within the range of 70–120%. The recoveries of seven pesticides (acephate, acrinathrin, chlorfluazuron, methamidophos, nitenpyram, oxycarboxine, and pyrimidifen) were below 70% in both samples. The low recoveries of chlorfluazuron, oxycarboxine, and pyrimidifen could be explained by low recoveries from the tandem graphitized carbon/PSA column, but the low recoveries of acephate, methamidophos, and nitenpyram were presumably caused by their high polarities, meaning that they preferentially partitioned into the aqueous phase at the salting-out step. The peak area ratios of matrix-matched standards to standards in solvent were 0.80–1.20, except for acrinathrin (0.70) and chlorfluazuron (0.61) in the cucumber samples, indicating that, for almost all of the pesticides, there were no significant matrix effects. The poor recoveries of acrinathrin from both sample types were presumably caused by ion suppression. All of the pesticides were successfully analyzed without serious interference from other peaks.

Although further work is needed to determine whether the developed SFE method can be applied to foods containing large amounts of matrix components, such as tea and beans, the results described above suggested that the developed SFE method can be used to efficiently extract pesticides having a wide polarity range from vegetable and fruit samples and is superior to the Japanese official method for extracting polar pesticides.

Table 2. Pesticide residue concentrations determined in unfortified samples by the supercritical fluid extraction (SFE) method and a liquid extraction method

Sample	Pesticide	Concentration (mg/kg, $n = 3$)				Ratio[a]	Japanese MRL (mg/kg)
		SFE		Liquid extraction			
		Ave.	RSD (%)	Ave.	RSD (%)		
Tomato 1	Boscalid	0.031	11	0.034	10	0.91	5
	Buprofezin	0.038	11	0.036	9	1.06	1
	Cyazofamid	0.017	5	0.018	4	0.94	2
Tomato 2	Mepanipyrim	0.020	5	0.019	12	1.05	5
Lemon	Chlorpyrifos	0.022	10	0.024	7	0.92	1
Spinach	Cyazofamid	0.61	4	0.79	1	0.77	25
Apple	Boscalid	0.022	9	0.021	8	1.05	3
	Propargite	0.16	10	0.13	4	1.23	3
	Pyraclostrobin	0.013	12	0.011	9	1.18	1
Cucumber	Acetamiprid	0.038	4	0.040	10	0.95	2

[a] SFE/Liquid extraction

4. Analysis of incurred residues in real samples

Concentrations of incurred residues in real samples obtained by the SFE method were compared with those by the Japanese official (liquid extraction) method. Six commercial vegetable and fruit samples were analyzed in triplicate using each method, and the results are shown in Table 2. Ten pesticides were detected (with log P_{ow} values of 0.8–5.7), and the RSDs of the concentrations were less than 12% for both methods. All of the pesticide concentrations were lower than the Japanese maximum residue levels (MRLs). The concentration ratios of the pesticide residues extracted by the SFE to those obtained by the liquid extraction method were 0.9–1.1, except for cyazofamid (0.77) in spinach, and pyraclostrobin (1.22) and propargite (1.18) in apple, suggesting that there were no significant differences in the extraction efficiencies between the two methods, regardless of the pesticide polarity. These results show that the SFE method we developed is suitable for the analysis of pesticide residues in real samples.

Conclusion

We developed a method for the simultaneous determination of LC-amenable polar and medium-polarity pesticides in vegetables and fruits using SFE and LC-MS/MS. The method involves supercritical CO_2 extraction of a 1 : 1 : 1 sample–Celite–anhydrous magnesium sulfate mixture using methanol added as a modifier followed by LC-MS/MS analysis. High recoveries were achieved for most of the tested pesticides. Our results indicate that SFE can be successfully applied to the determination of not only GC-amenable pesticides but also LC-amenable polar and medium-polarity pesticides.

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Simultaneous determination of acidic pesticides in vegetables and fruits by liquid chromatography—tandem mass spectrometry

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A sensitive and efficient method has been developed for the simultaneous determination of 73 multi-class acidic pesticides, such as phenoxy acid and sulfonylurea herbicides, in vegetables and fruits. The sample preparation procedure was carefully optimized for the efficient removal of co-extracted matrix components. The method involves extraction of acidic pesticides with acetonitrile containing hydrochloric acid, removal of water from crude extract by salting out, and sequential cleanup by octadecylsilyl silica gel and silica gel columns. For samples containing high amounts of pigments, such as spinach, additional cleanup using a graphitized carbon column was performed prior to liquid chromatography–mass spectrometry (LC–MS/MS) analysis. Recovery tests were performed for five times for each sample of cabbage, spinach, potato, eggplant, orange, and apple fortified at 0.01 mg kg−1. Out of the 73 tested pesticides, 70 for cabbage, 67 for spinach, 69 for potato, 67 for eggplant, 64 for orange, and 70 for apple were within the range of 70–120%, with relative standard deviations below 25%. Nitenpyram and pyrasulfotole showed low recoveries for all the samples tested, probably due to low recoveries from silica gel column. The developed method effectively removed co-extracted matrix components and was highly selective, with no interfering peaks found in the chromatograms of blank samples. The overall results indicate that the developed method is suitable for the quantitative analysis of acidic pesticide residues in vegetables and fruits.

Keywords: Acidic pesticides, LC–MS/MS, Multi-residue method, vegetables and fruits.

Introduction

Pesticides are used extensively throughout the world to protect agricultural products from pests such as insects, bacteria, fungi, and viruses. However, since pesticides are harmful to humans as well as animals and the environment,[1,2] pesticide residues must be controlled. Therefore, regulatory agencies of many countries have established maximum residue limits (MRLs) in foods, with the aim of minimizing the health risks associated with their consumption. For this reason, it is important to develop sensitive and reliable Multi-residue methods for monitoring pesticides in various foods. To date, numerous Multi-residue methods have been developed for monitoring pesticides in foods. Anastassiades et al. reported the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for the analyses of a wide range of pesticides;[3] many modified versions of this method applied to various foods have been subsequently reported.[4–7] Fillion et al. demonstrated a Multi-residue method in vegetables and fruits by employing gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC) with fluorescence detection.[8] In Japan, an official "Multi-residue method I for agricultural chemicals by LC–MS" has been established (Fig. 1a),[9] which is a modification of the method developed by Fillion et al.[8] These methods use anion exchange sorbents – i.e., primary secondary amine (PSA) or aminopropyl-silanized silica gel (NH_2) sorbents – for cleanup and can effectively remove acidic co-extracted matrix components such as organic and fatty acids. However, PSA and NH_2 sorbents also retain acidic pesticides, leading to poor recoveries.

Several methods have been published for the analysis of phenoxy acid herbicides in vegetables and fruits,[10,11] rice,[12] and kidney tissues.[13] In addition, methods have been reported for analyzing sulfonylurea herbicides in crops,[14–16] grapes,[17] and milk.[18] However, the simultaneous determination of multi-class acidic pesticides in complicated food matrices is a challenging task because of the wide range of pK_a values and polarities of pesticides; consequently, there are very few reported methods for such analyses. Pareja et al. have evaluated various

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