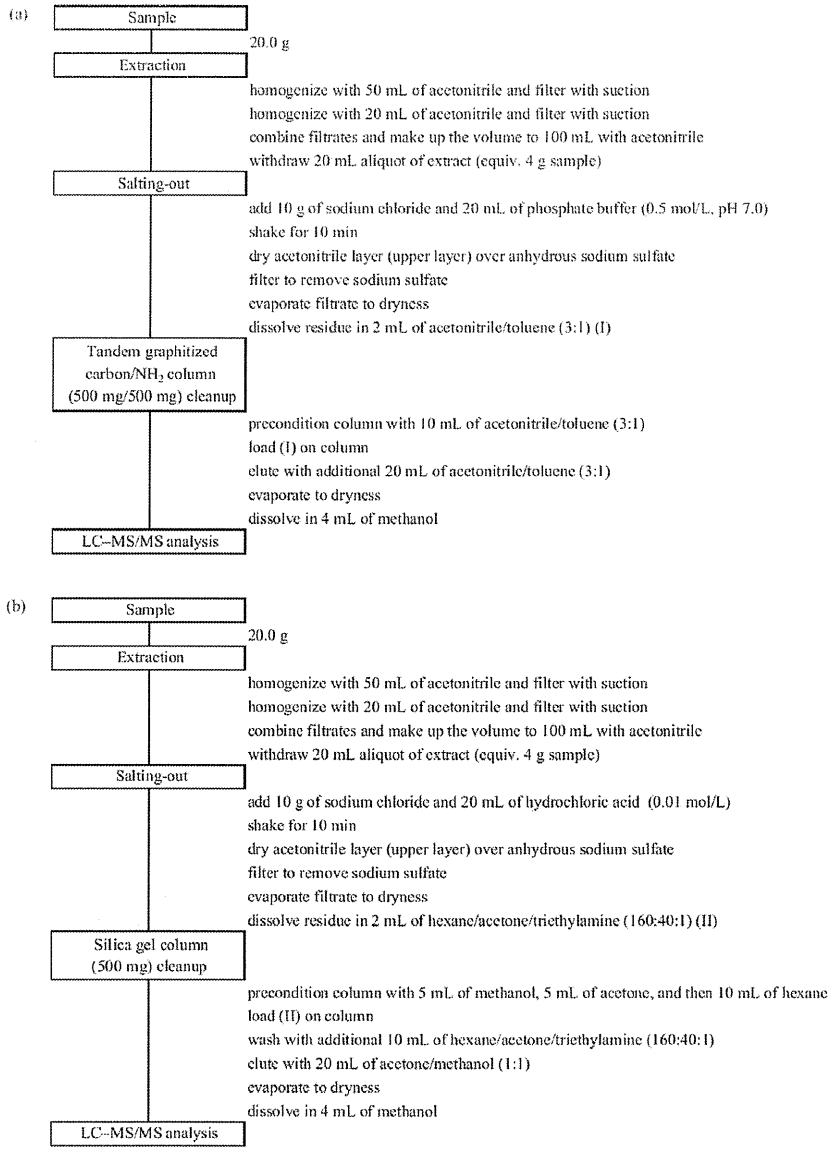

Fig. 1. Flow chart showing the sequence of steps in (a) the Japanese official Multi-residue method I, and (b) the Japanese official Multi-residue method II in vegetables and fruits.

modified QuEChERS methods and described a method with no cleanup step for analyzing acidic herbicides, including phenoxy acid and sulfonylurea herbicides, in polished rice.[19] Akiyama et al. demonstrated the simultaneous determination of multi-class acidic pesticides in vegetables and fruits without a cleanup step and determined by liquid chromatography time-of-flight mass spectrometry (LC–TOFMS).[20] In Japan, an official "Multi-residue method II for agricultural chemicals by LC–MS" has been established (Fig. 1b)[9] for acidic pesticides that cannot be determined by the Japanese official Multi-residue method I (Fig. 1a). However, the removal of co-extracted matrix components without using PSA or NH_2 sorbent is insufficient, often causing significant matrix effects.

The aim of this study was to develop a sensitive and reliable Multi-residue method for acidic pesticides in

vegetables and fruits by modifying the official Multi-residue method II (Fig. 1b) implemented in Japan. The sample preparation procedure was carefully optimized to develop a highly efficient method for the removal of co-extracted matrix components. The newly developed method was applied for the simultaneous determination of 73 multi-class acidic pesticides in vegetables and fruits.

Experimental

Reagents and materials

Solvents and chemicals. Pesticide-analysis-grade acetone, acetonitrile, hexane, methanol, and toluene were obtained from Kanto Chemical (Tokyo, Japan). Pesticide-analysis-grade sodium chloride and analytical-grade ammonium sulfate, formic acid, and 0.1 mol L^{-1} hydrochloric acid were purchased from Wako Pure Chemical (Osaka, Japan). LC–MS-grade methanol and water were obtained from Kanto Chemical and used for LC–MS/MS analyses. Diatomaceous earth (Celite®, No. 545) was obtained from Wako Pure Chemical. Water used to prepare test solutions was purified in an NZJ-2DSYW distillation apparatus (Fujiwara Scientific, Tokyo, Japan).

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^{-1}) were prepared in acetonitrile or methanol, depending on the solubility of 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 working standard solutions with methanol.

Cartridge columns. Octadecylsilyl silica gel (ODS) columns (Mega Bond Elut C18, 1,000 mg) were obtained from Agilent Technologies (Palo Alto, CA, USA), and graphitized carbon (InertSep GC, 500 mg) and silica gel (InertSep SI, 1 g) columns were obtained from GL Sciences (Tokyo, Japan).

Apparatus

LC–MS/MS. An Acquity UPLC system (Waters, Milford, MA, USA) coupled with a Xevo TQ-S mass spectrometer (Waters) was used, with the following operating conditions: Inertsil ODS-4 column (length: 150 mm, i.d.: 2.1 mm, particle size: 3 μm; GL Sciences); mobile phases, 5 mmol L^{-1} ammonium acetate in water (Solvent A) and 5 mmol L^{-1} ammonium acetate in methanol (Solvent B); solvent gradient, 10% Solvent B at 0 min, 95% Solvent B at 20 min, 95% Solvent B at 30 min, 100% Solvent B at 30.1 min, 100% Solvent B at 40 min, 10% Solvent B at 40.1 min; flow rate, 0.2 mL min^{-1}; column temperature, 40°C; injection volume, 5 μL; ionization mode, electrospray ionization (ESI); capillary voltage, 3 kV (ESI (+)), 1 kV (ESI(−)); source temperature, 150°C; desolvation temperature, 500°C; desolvation gas, nitrogen at 1,000 L h^{-1}; cone gas, nitrogen at 150 L h^{-1}; and collision gas, argon at 0.15 mL min^{-1}. The selective reaction monitoring (SRM) transitions and retention times are summarized in Table A1.

Laboratory apparatus. A homogenizer (Polytron PT 10-35 GT; Kinematica, Lucerne, Switzerland), food processor (Grindomix GM 200; 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) were connected to a Kiriyama funnel containing filter paper No. 5B (Kiriyama Glass Works, Tokyo, Japan).

Sample preparation

Foods containing low amounts of pigments. A 20.0-g sample was weighed in a 250-mL glass tube, and was extracted with 40 mL of acetonitrile and 10 mL of 0.1 mol L^{-1} hydrochloric acid by using a homogenizer. The homogenate was filtered under vacuum through Celite® filter aid, and the residue was re-homogenized with 20 mL of acetonitrile and filtered again. The volume of the combined extract was made up to 100 mL with acetonitrile.

A 10-mL aliquot of the extract was added to a 50-mL PTFE centrifuge tube containing 5 g of sodium chloride and shaken vigorously for 10 min. The extract was then centrifuged at 3,000 rpm for 5 min. The resultant acetonitrile layer was loaded on an ODS column preconditioned with 10 mL of acetonitrile and eluted with an additional 5 mL of acetonitrile. The combined eluate was concentrated to approximately 1 mL with a rotary evaporator below 40°C and evaporated to dryness under a stream of nitrogen.

The residue was subsequently re-dissolved in 2 mL of hexane/acetone/formic acid (100:100:1 v/v/v) and loaded on a silica gel column (1 g), eluting with an additional 18 mL of hexane/acetone/formic acid (100:100:1 v/v/v). The combined eluate was concentrated to approximately 1 mL with a rotary evaporator below 40°C, evaporated to dryness under a stream of nitrogen, and the residue was re-dissolved in 4 mL of methanol.

For foods containing high amounts of pigments (spinach). Samples were prepared using the same procedure as mentioned above. The residue obtained after silica gel column cleanup was re-dissolved in 2 mL of acetonitrile/toluene/water (30:10:1 v/v/v), and subsequently loaded onto a

graphitized carbon column (500 mg) and eluted with an additional 23 mL of acetonitrile/toluene/water (30:10:1 v/v/v). The combined eluate was concentrated to approximately 1 mL with a rotary evaporator below 40°C and evaporated to dryness under a stream of nitrogen; the obtained residue was re-dissolved in 4 mL of methanol.

Recovery tests

The recovery tests were performed for cabbage, spinach, potato, eggplant, orange, and apple, with five replicates for all matrices at a spiking level of 0.01 mg kg^{-1}. A 1-mL aliquot of working standard solution was added to the samples and were allowed to stand for 30 min before extraction. Quantifications were carried out using six points (12.5, 25, 50, 75, 100, 150%) from external calibration using solvent standards.

Preparation of matrix-matched standards. Matrix-matched standards were prepared as follows: Blank extract solutions of 100 μL were evaporated to dryness under a stream of nitrogen, and the residue was re-dissolved in 100 μL of 0.005 μg mL^{-1} standard mixture (0.0005 μg mL^{-1} for warfarin) in methanol.

Results and discussion

Selection of pesticides and optimization of LC–MS/MS conditions

In this study, a total of 73 LC-amenable acidic pesticides, mostly herbicides with a wide pK_a range,[2–6] were selected (Table A1). These pesticides are retained on PSA or NH_2 sorbent when using acetonitrile/toluene (3:1 v/v) as elution solvent, and could not be determined by the official Japanese Multi-residue method I (Fig. 1a).

Optimizations of LC–MS/MS conditions were performed by flow injection analysis of individual standards. The precursor and product ions were optimized by varying cone voltage in the range of 10–80 V and collision energy in the range of 5–45 eV in both ESI(+) and ESI(−) modes. The most intense transition was used for quantification, and the second transition was used for confirmation (Table A1).

Figure 2 shows representative chromatograms of standards, blank apple sample, and fortified sample. Satisfactory separations and peak shapes were achieved for most of the tested pesticides by gradient elution using methanol and water containing 5 mmol L^{-1} of ammonium acetate, except for highly polar pesticides with short retention time (i.e. mesotrione, picloram, and trinexapac), which gave tailing peaks.

Optimization of sample preparations

To develop a reliable and rugged Multi-residue method that can sufficiently extract acidic pesticides having a wide range of pK_a values from foods, regardless of their pH, the pH of extraction should be controlled.[12] In addition, homogenization with a probe blender is known to accelerate extraction more significantly than shaking.[21] Thus, in this study, 0.1 mol L^{-1} hydrochloric acid was added to acetonitrile, and extraction was performed by homogenizing with a probe blender.

To remove water from the crude extract by salting out, 5 g of sodium chloride was added to 10 mL of crude extract and shaken for 10 min. Since a mixture of hydrochloric acid and acetonitrile was used for extraction in this study, the pH values of aqueous (lower) layers after the salting-out step are as follows: cabbage, 3.3; spinach, 2.2; potato, 3.4; eggplant, 2.3; orange, 2.2; and apple, 2.1. All pesticides were partitioned into the acetonitrile layer in all the tested foods, although the recoveries of seven pesticides out of 73 were low under neutral conditions (pH 7). It seems that in this pH range most acidic pesticides exist in their neutral forms; thus, it was unnecessary to add additional acid at the salting-out step.

The presence of low-polarity matrix components during determination would be troublesome, since they affect the LC–MS/MS performance and cause matrix effects; therefore, these components should be sufficiently removed prior to LC–MS/MS analysis. Thus, in this study, ODS column cleanup was added after the salting-out step. The acetonitrile (upper) layer obtained after salting out was directly loaded on an ODS column and eluted with an additional 5 mL of acetonitrile. Low-polar matrix components, such as green pigments (chlorophylls), were retained on the column, while all the tested pesticides were eluted with high recoveries.

Since the target pesticides of this study ranged from relatively weak to strong acids, a silica gel column was used for further cleanup instead of a PSA or NH_2 anion exchange column, as in the Japanese official Multi-residue method II. As shown in Fig. 1b, the silica gel column cleanup procedure in the official Multi-residue method II involves sample loading with 2 mL of acetone/hexane/triethylamine (20:80:0.5 v/v/v), washing with an additional 10 mL of the same solvent mixture to remove low-polarity matrices, and then elution of pesticides with 10 mL of acetone/methanol (1:1 v/v). However, in this study, we omitted the washing procedure with acetone/hexane/triethylamine (20:80:0.5 v/v/v) because low-polarity pesticides elute together with low-polarity matrix components. Instead, we added the above-mentioned ODS column cleanup procedure to remove these matrices. Figure 3 shows the matrix effects of the representative pesticides in orange by eluting with various solvents from the silica gel column. Although eluting with acetone/methanol (1:1 v/v), the elution solvent used in the Japanese

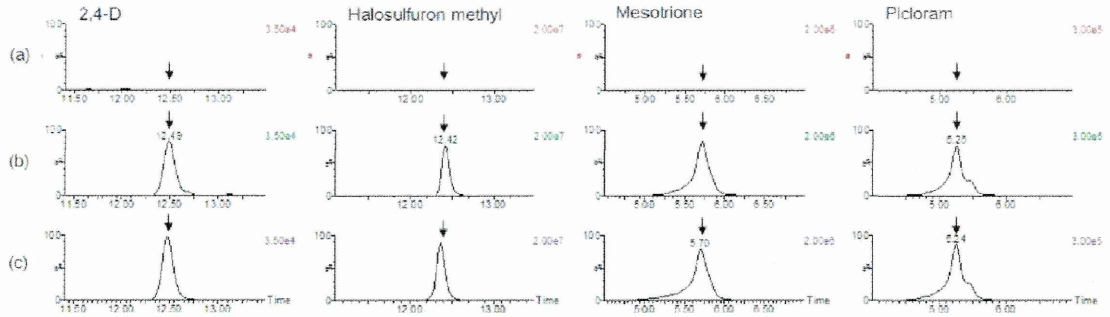

Fig. 2. Representative chromatograms of (a) extract of blank apple, (b) extract of apple spiked with 0.01 mg kg^{-1}, and (c) 0.005 μg mL^{-1} standard solutions.

official Multi-residue method II achieved high recoveries of tested pesticides, large amounts of high-polarity components eluted along with the target pesticides, causing a significant matrix effect. In contrast, acetone alone eluted a small amount of matrix components from the silica gel column but yielded low recoveries of tested pesticides. Addition of formic acid to acetone improved pesticide recoveries but eluted matrix components and showed significant matrix effects. In contrast, addition of hexane, a lower-polarity solvent, to acetone/formic acid resulted in considerably decreased matrix effects for most of the target pesticides. Thus, by varying the proportion of acetone, hexane, and formic acid, the elution solvent was optimized. As a result, acetone/hexane/formic acid (100:100:1 v/v/v) has been shown to be an optimal elution solvent, giving minimal matrix effects with satisfactory recoveries except for mesotrione, nitenpyram, pyrasulfotole, and trinexapac. Although most of the pesticides were eluted with 10 mL of acetone/hexane/formic acid (100:100:1 v/v/v), foramsulfuron required 20 mL to elute. Thus, 20 mL of acetone/hexane/formic acid (100:100:1 v/v/v) was considered to give appropriate elution conditions for silica gel column cleanup.

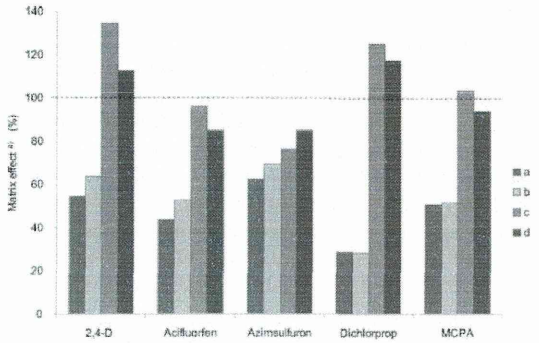

Fig. 3. Effect of elution solvents from silica gel column on matrix effects[a] of pesticide peak areas using an orange sample: a: methanol/acetone (1:1), 10 mL; b: acetone/formic acid (200:1), 10 mL; c: hexane/acetone/formic acid (50:150:1), 20 mL; d: hexane/acetone/formic acid (100:100:1), 20 mL. [a]Peak area ratio of a matrix-matched standard to standard in a pure solvent.

Although the combination of ODS and silica gel column cleanup has been shown to be very effective for vegetables and fruits, the removal of pigments was not sufficient for samples containing high amounts of pigments, especially for spinach. Many methods for vegetables have been published using graphitized carbon sorbents for removing pigments.[8,9,22] However, for acidic pesticides, few methods using graphitized carbon have been reported, since acidic pesticides are prone to retention on the graphitized carbon sorbent. This can be explained by the anion exchange properties of positively charged oxonium groups, hydrophobic interactions between graphite carbon and the aromatic structure of pesticides, and hydrogen bonding between protonated functional groups of pesticides and carbonyl groups of the graphitized carbon or vice versa.[23] However, because graphitized carbon effectively removes pigments, we attempted to elute acidic pesticides from a graphitized carbon column by examining various solvents. Recoveries of representative pesticides from graphitized carbon column using different elution solvents are shown in Fig. 4. By using solvent standards, five pesticides (cyclanilide, flusulfamide, forchlorfenuron, pyrasulfotole, and tecloftalam) out of 73 showed poor recoveries (<60%) by eluting with 22 mL of acetonitrile/toluene (3:1 v/v), which are the elution conditions used in the Japanese official Multi-residue method I. Recoveries of the tested pesticides changed slightly by varying the ratio of acetonitrile and toluene to (9:1 v/v) or (1:1 v/v). Moreover, the recoveries of most of the pesticides were affected only minimally by the addition of formic acid to acetonitrile/toluene (3:1 v/v). In contrast, addition of water to acetonitrile/toluene (3:1 v/v) gave significantly better recoveries. Thus, the ratio of water was optimized by comparing the recovery of

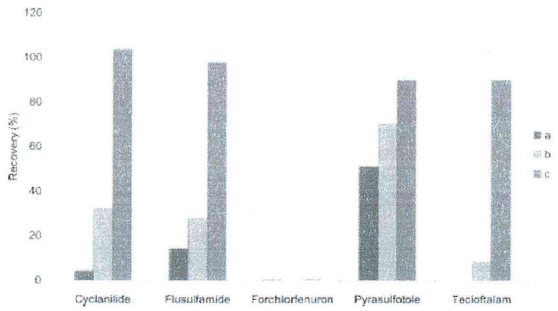

Fig. 4. Effect of elution solvents on the recoveries of pesticides from graphitized carbon column: a: acetonitrile/toluene (3:1), 20 mL; b: acetonitrile/toluene (1:1), 20 mL;°C: acetonitrile/toluene/water (30:10:1), 20 mL.

pesticides from the graphitized carbon column with an acetonitrile/toluene/water eluting solvent of volume compositions of 30:10:0.2 v/v/v, 30:10:1 v/v/v, and 30:10:2 v/v/v. Considerably higher recoveries were obtained by eluting with acetonitrile/toluene/water 30:10:1 v/v/v compared with 30:10:0.2 v/v/v, while no great difference in recoveries was observed between eluting with acetonitrile/toluene/water 30:10:1 v/v/v and 30:10:2 v/v/v. Most of the tested pesticides showed sufficient recoveries with 15 mL of acetonitrile/toluene/water (30:10:1 v/v/v), but flusulfamide and tecloftalam required 25 mL to elute from the column. Only forchlorfenuron (0%) and trinexapac (66%) showed poor recoveries by eluting with 25 mL of acetonitrile/toluene/water (30:10:1 v/v/v). Therefore, 25 mL of acetonitrile/toluene/water (30:10:1 v/v/v) was considered to be the optimal elution solvent composition for use with the graphitized carbon column and can achieve high recoveries for most of the tested pesticides. The additional graphitized carbon column cleanup procedure effectively removed pigments, yielding a colorless solution from the spinach extract. The overall scheme of the above-described analytical procedure is shown in Fig. 5.

Recovery test

The recovery tests were performed for five times for each sample of cabbage, spinach, potato, eggplant, orange, and

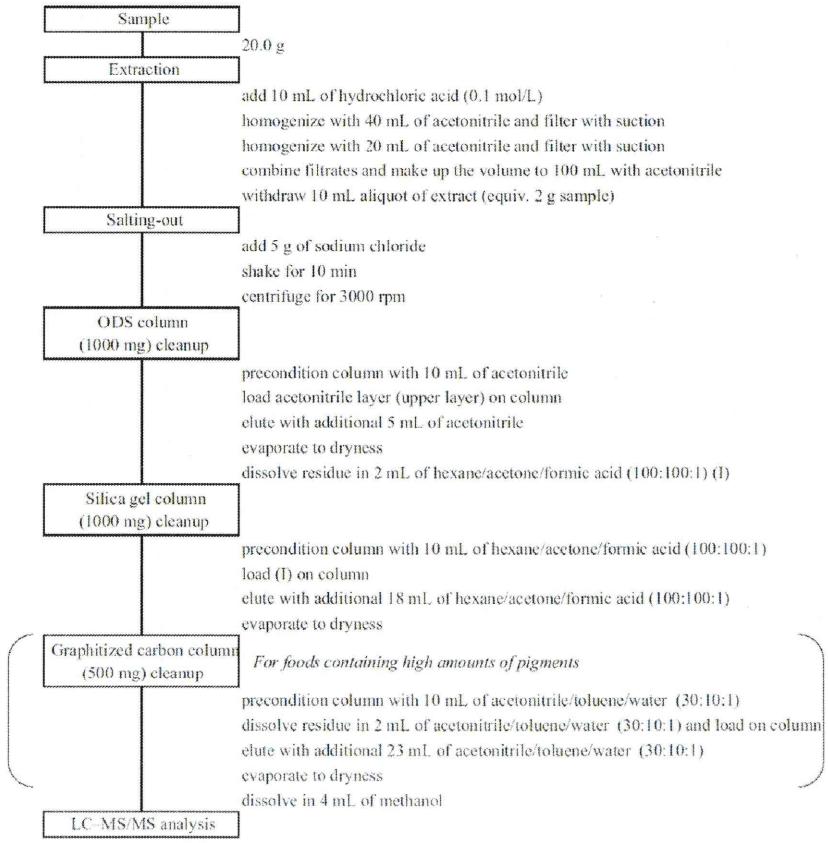

Fig. 5. Flow chart showing the sequence of steps in the developed multi-residue method.

Table 1. Recoveries of pesticides from fortified vegetables and fruits.

	Cabbage		*Spinach*		*Potato*		*Eggplant*		*Orange*		*Apple*	
	Recovery (%)	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*
2,4-D	84	6	76	6	88	7	85	10	87	7	87	11
2,4-DB	95	5	75	23	77	8	73	12	94	17	93	14
4-CPA	84	10	70	13	91	16	82	12	60	14	94	14
Acifluorfen	95	4	72	11	82	12	73	12	83	10	88	11
Azimsulfuron	84	2	85	3	87	1	80	3	81	4	93	2
Bensulfuron methyl	93	1	88	3	97	1	93	3	99	3	89	2
Bentazone	85	3	77	6	86	5	86	5	21	22	85	7
Bispyribac sodium	83	2	82	2	94	1	94	3	91	2	82	2
Bromoxynil	94	5	80	13	76	7	75	4	83	10	89	9
Bupirimate	87	1	84	2	87	2	84	3	81	2	77	3
Chlorimuron ethyl	88	2	84	4	95	1	83	2	102	3	85	3
Chlorsulfuron	86	1	83	4	88	2	88	3	96	1	88	2
Cinosulfuron	82	2	89	3	92	1	90	4	105	2	94	1
Clodinafop acid	80	1	86	2	72	3	72	4	91	2	79	3
Cloprop	91	5	74	23	87	12	78	16	101	16	91	18
Cloransulam methyl	92	2	90	5	101	2	92	3	114	3	100	2
Cyclanilide	99	3	75	13	72	6	84	6	87	9	89	7
Cyclosulfamuron	89	2	87	2	91	2	83	3	79	3	87	3
Dichlorprop	83	9	62	16	100	10	102	5	86	8	88	10
Diclosulam	88	1	88	3	94	1	90	2	81	2	85	2
Ethametsulfuron methyl	87	3	84	3	91	2	82	3	92	3	89	2
Ethoxysulfuron	84	2	83	3	89	1	81	3	83	2	81	3
Fenoprop	103	6	81	6	75	3	96	8	99	8	88	9
Flazasulfuron	83	2	79	2	75	4	71	2	81	3	76	2
Florasulam	90	0.4	86	4	89	1	81	1	114	1	83	1
Fluazifop	92	3	72	2	74	3	85	2	97	1	85	2
Flucarbazone sodium	91	1	87	4	100	1	86	2	96	3	92	3
Flucetosulfuron	95	1	112	4	116	1	86	3	88	1	101	2
Flumetsulam	92	1	88	4	92	1	73	3	86	2	87	2
Flusulfamide	75	9	85	7	84	3	76	4	62	4	81	8
Fomesafen	89	3	78	2	75	2	79	9	74	3	85	3
Foramsulfuron	83	2	83	4	89	0.5	75	4	93	3	78	4
Forchlorfenuron	89	2	14	17	84	3	29	3	74	1	71	2
Gibberellic acid	78	2	76	4	81	5	81	4	66	3	83	6
Halosulfuron methyl	74	2	83	4	86	1	64	1	77	3	72	4
Haloxyfop	87	2	83	2	91	5	82	3	73	2	87	3
Imazamethabenz methyl	84	1	84	1	76	2	79	3	70	3	87	1
Imazaquin	85	2	82	3	82	3	86	2	70	3	87	3
Imazosulfuron	80	2	74	4	72	3	71	5	94	4	81	2
Iodosulfuron methyl	89	1	91	3	106	2	91	3	93	1	77	4
Ioxynil	85	4	73	3	80	3	82	8	50	17	91	4
MCPA	89	4	86	18	95	8	89	10	102	8	77	5
Mecoprop	77	5	84	21	96	11	101	11	81	15	89	7
Mesosulfuron methyl	84	2	93	4	99	1	85	4	119	2	92	2
Mesotrione	78	2	76	5	74	3	80	2	103	2	92	2
Metosulam	87	1	93	5	97	2	77	1	107	3	91	2
Metsulfuron methyl	81	2	88	4	99	1	99	3	119	3	89	3
Naptalam	72	1	71	4	73	2	73	1	75	6	72	3
Nitenpyram	0		0		0		0		0		0	
Oryzalin	89	3	84	2	75	6	71	3	70	2	85	4
Penoxsulam	92	1	94	3	101	2	92	1	105	2	97	2
Picloram	78	3	58	2	60	3	82	6	79	11	83	2

(*Continued on next page*)

Table 1. Recoveries of pesticides from fortified vegetables and fruits. *(Continued)*

	Cabbage		*Spinach*		*Potato*		*Eggplant*		*Orange*		*Apple*	
	Recovery (%)	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*
Primisulfuron methyl	72	8	88	20	109	10	82	10	76	8	81	8
Propoxycarbazone	92	1	88	2	91	1	83	2	114	0.4	97	2
Propyrisulfuron	75	2	85	3	94	1	88	3	80	2	83	3
Prosulfuron	83	6	86	2	97	2	79	3	96	4	76	4
Pyrasulfotole	0	—	0	—	0	—	0	—	0	—	0	—
Pyrazosulfuron ethyl	83	1	84	4	88	1	80	2	92	3	74	5
Pyrithiobac sodium	89	3	77	2	75	2	85	3	87	1	73	4
Quinclorac	79	3	70	3	70	3	75	3	73	5	84	1
Rimsulfuron	80	2	84	3	83	4	82	4	83	4	85	2
Sulfosulfuron	90	2	86	3	75	2	82	3	96	3	92	3
Tecloftalam	36	13	50	16	47	5	43	8	41	10	76	6
Thifensulfuron methyl	78	1	90	3	99	1	99	3	110	2	96	1
Tralkoxydim	79	4	83	3	70	4	85	4	79	4	81	3
Triasulfuron	94	1	85	4	95	1	90	3	96	2	91	2
Tribenuron methyl	77	6	86	3	78	7	43	7	58	8	47	8
Triclopyr	90	7	104	10	78	7	83	8	81	7	73	12
Trifloxysulfuron	84	3	87	3	88	5	78	4	90	5	77	5
Triflusulfuron methyl	104	3	87	3	97	2	96	4	96	3	91	4
Trinexapac	95	6	71	5	73	1	75	3	77	4	92	4
Trinexapac ethyl	84	2	80	2	81	4	73	1	86	5	86	1
Warfarin[a]	87	2	78	5	77	1	83	6	76	1	88	3

[a]Fortified at 0.001 mg kg^{-1}.

apple fortified at 0.01 mg kg^{-1}, except for warfarin at 0.001 mg kg^{-1}. A spike level of 0.01 mg kg^{-1} was chosen because, in Japan, a uniform limit of 0.01 mg kg^{-1} is applied to all pesticides for which MRLs are not established. In addition, MRLs of warfarin have been set at 0.001 mg kg^{-1} in various foods. Although matrix-matched calibration is often used to compensate for matrix effects, it is not necessarily accurate in real-world analyses because matrix effects vary with different blank samples. Thus, external calibration using solvent standards, instead of matrix-matched calibration, was used for quantifications. The results of the recovery tests are shown in Table 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 70–120%, with relative standard deviations below 25%, and were within the acceptable range of the Japanese guideline value.[24] Nitenpyram and pyrasulfotole were not recovered in all the samples tested. A possible reason for low recoveries of nitenpyram and pyrasulfotole would be their low recovery from the silica gel column, while poor recoveries of forchlorfenuron for the spinach sample can be explained by its insufficient elution from the graphitized carbon column. Trinexapac and mesotrione achieved high recoveries, in spite of poor recoveries in pure solvents from the silica gel column, indicating that coexisting matrix components facilitated the elution of these pesticides. The matrix effects were evaluated by comparing the peak areas of matrix-matched standards with those of the standards in solvent (Table A2). Although some pesticides, such as picloram, tecloftalam, and tribenuron methyl, gave poor recoveries by ion suppression, the test solutions obtained by the developed method were much cleaner compared with those obtained by the Japanese official Multi-residue method II. A satisfactory linearity for all the tested pesticides was obtained with a correlation coefficient of >0.995. No significant interfering peak was observed in all blank samples, which indicated high selectivity of the developed method.

Conclusions

In this study, we developed a sensitive and reliable LC–MS/MS method for the determination of a wide range of acidic pesticides in vegetables and fruits by modifying the Japanese official Multi-residue method II. The combination of ODS, silica gel, and graphitized carbon column cleanups effectively removed co-extracted matrix components and was suitable for subsequent LC–MS/MS analysis. Recoveries and precisions of most of the 73 tested pesticides were within the acceptable range of the Japanese guideline. Overall, the results suggest that the newly developed method is suitable for routine monitoring of acidic pesticide residues in vegetables and fruits.

Funding

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Appendix

Table A1. LC-MS/MS parameters for the tested pesticides.

			Quantification				*Confirmation*			
	Ionization mode	*Retention time (min)*	*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)*
2,4-D	ESI(−)	12.5	219.0	160.9	30	10	219.0	125.1	30	25
2,4-DB	ESI(−)	15.3	160.8	124.9	40	15	247.1	161.0	60	15
4-CPA	ESI(−)	9.9	185.0	127.0	40	15	187.0	127.0	40	15
Acifluorfen	ESI(−)	16.1	360.1	316.0	10	10	360.1	195.0	10	25
Azimsulfuron	ESI(+)	9.8	425.3	182.2	50	20	425.3	156.2	50	35
Bensulfuron methyl	ESI(+)	15.0	411.3	149.2	50	20	411.3	182.2	50	20
Bentazone	ESI(−)	8.5	239.1	132.1	60	25	239.1	197.0	60	20
Bispyribac sodium	ESI(+)	15.1	431.3	275.2	30	15	431.3	413.2	30	15
Bromoxynil	ESI(−)	10.8	275.9	80.9	60	25	275.9	78.9	60	25
Bupirimate	ESI(+)	19.4	317.3	166.2	50	25	317.3	150.2	50	20
Chlorimuron ethyl	ESI(+)	13.3	415.2	186.1	40	20	415.2	121.1	40	40
Chlorsulfuron	ESI(+)	9.9	358.2	141.2	40	15	358.2	167.2	40	15
Cinosulfuron	ESI(+)	9.9	414.3	183.2	50	15	414.3	157.2	50	25
Clodinafop acid	ESI(+)	14.6	312.2	266.2	50	15	312.2	91.1	50	25
Cloprop	ESI(−)	11.3	199.0	127.0	10	15	199.0	71.0	10	10
Cloransulam methyl	ESI(+)	13.3	430.2	398.1	40	15	430.2	370.1	40	20
Cyclanilide	ESI(−)	14.8	272.0	160.0	40	20	272.0	228.0	40	10
Cyclosulfamuron	ESI(+)	16.0	422.3	261.2	40	15	422.3	218.2	40	25
Dichlorprop	ESI(−)	14.0	233.0	161.0	10	15	233.0	125.0	10	25
Diclosulam	ESI(+)	13.6	406.1	161.1	40	25	406.1	378.1	40	15
Ethametsulfuron methyl	ESI(+)	11.7	411.3	196.2	40	15	411.3	168.2	40	30
Ethoxysulfuron	ESI(+)	13.6	399.2	261.2	50	15	399.2	218.2	50	25
Fenoprop	ESI(−)	15.7	266.8	194.7	10	15	266.8	158.7	10	30
Flazasulfuron	ESI(+)	10.7	408.2	182.2	40	20	408.2	139.2	40	40
Florasulam	ESI(+)	9.8	360.2	129.2	40	20	360.2	192.1	40	15
Fluazifop	ESI(+)	14.6	328.2	282.2	50	20	328.2	254.2	50	25
Flucarbazone sodium	ESI(+)	10.4	397.2	130.2	30	10	397.2	115.2	30	40
Flucetosulfuron	ESI(+)	11.3	488.3	156.2	40	20	488.3	273.2	40	25
Flumetsulam	ESI(+)	7.5	326.2	129.2	50	25	326.2	262.2	50	20
Flusulfamide	ESI(−)	18.2	413.0	171.0	80	35	413.0	349.0	80	25
Fomesafen	ESI(+)	16.1	456.2	344.1	30	15	456.2	223.2	30	30
Foramsulfuron	ESI(+)	10.2	453.3	182.2	30	20	453.3	272.2	30	10
Forchlorfenuron	ESI(+)	16.4	248.2	129.1	40	20	248.2	93.1	40	30
Gibberellic acid	ESI(+)	8.3	364.3	239.3	30	15	364.3	311.3	30	15
Halosulfuron methyl	ESI(+)	12.4	435.2	182.2	40	20	435.2	139.2	40	40
Haloxyfop	ESI(+)	16.7	362.2	288.2	40	25	362.2	316.2	40	15
Imazamethabenz methyl	ESI(+)	14.7	289.3	144.1	50	35	289.3	161.2	50	25
Imazaquin	ESI(+)	9.7	312.3	267.2	40	20	312.3	199.2	40	25
Imazosulfuron	ESI(+)	11.0	413.2	153.2	40	10	413.2	156.2	40	20
Iodosulfuron methyl	ESI(+)	12.2	508.1	167.2	40	20	508.1	141.2	40	25
Ioxynil	ESI(−)	12.8	369.9	127.0	40	30	369.9	215.0	40	30
MCPA	ESI(−)	12.5	199.1	141.0	30	15	199.1	105.0	30	25
Mecoprop	ESI(−)	13.9	213.1	141.0	30	20	213.1	71.1	30	10
Mesosulfuron methyl	ESI(+)	11.4	504.2	182.2	50	20	504.2	162.2	50	40
Mesotrione	ESI(+)	5.7	340.2	228.1	40	15	340.2	104.1	40	30
Metosulam	ESI(+)	12.9	418.2	175.2	50	25	418.2	140.2	50	40
Metsulfuron methyl	ESI(+)	8.8	382.2	167.2	40	15	382.2	199.2	40	20

(*Continued on next page*)

Table A1. LC-MS/MS parameters for the tested pesticides. *(Continued)*

			Quantification				*Confirmation*			
	Ionization mode	*Retention time (min)*	*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)*
Naptalam	ESI(+)	12.1	292.3	144.1	30	10	292.3	149.1	30	20
Nitenpyram	ESI(+)	7.0	271.2	126.1	30	25	271.2	130.2	30	10
Oryzalin	ESI(+)	18.5	347.2	288.2	40	15	347.2	305.2	40	15
Penoxsulam	ESI(+)	12.9	484.2	195.2	60	25	484.2	164.2	60	35
Picloram	ESI(+)	5.2	241.1	168.0	30	30	241.1	195.0	30	20
Primisulfuron methyl	ESI(−)	14.8	467.1	226.1	10	15	467.1	176.1	10	30
Propoxycarbazone	ESI(+)	10.6	399.1	198.9	20	10	399.1	158.0	20	10
Propyrisulfuron	ESI(+)	13.6	456.2	261.2	40	15	456.2	196.2	40	15
Prosulfuron	ESI(+)	13.9	420.2	141.2	50	20	420.2	167.2	50	20
Pyrasulfotole	ESI(+)	10.7	363.2	251.1	50	25	363.2	220.1	50	40
Pyrazosulfuron ethyl	ESI(+)	12.1	415.2	182.2	40	25	415.2	139.2	40	40
Pyrithiobac sodium	ESI(+)	13.6	327.2	309.1	40	15	327.2	139.2	40	25
Quinclorac	ESI(+)	8.4	242.1	161.1	30	35	242.1	224.1	30	15
Rimsulfuron	ESI(+)	9.1	432.2	182.2	40	25	432.2	325.2	40	15
Sulfosulfuron	ESI(+)	10.7	471.2	211.2	40	15	471.2	261.2	40	15
Tecloftalam	ESI(+)	17.9	465.2	162.1	20	15	466.9	162.1	20	15
Thifensulfuron methyl	ESI(+)	8.9	388.2	167.2	40	15	388.2	205.1	40	25
Tralkoxydim	ESI(+)	15.4	330.4	284.3	40	10	330.4	138.2	40	20
Triasulfuron	ESI(+)	10.9	402.2	167.2	40	15	402.2	141.2	40	20
Tribenuron methyl	ESI(+)	11.1	396.2	155.2	40	10	396.2	181.2	40	20
Triclopyr	ESI(+)	13.4	256.1	146.0	30	25	257.9	146.0	30	25
Trifloxysulfuron	ESI(+)	12.2	438.2	182.2	30	25	438.2	139.2	30	40
Triflusulfuron methyl	ESI(+)	14.7	493.3	264.2	40	20	493.3	96.1	40	40
Trinexapac	ESI(+)	3.1	225.0	68.9	40	15	225.0	164.9	40	15
Trinexapac ethyl	ESI(+)	9.4	253.0	207.0	20	10	253.0	185.0	20	10
Warfarin	ESI(+)	12.9	309.3	163.2	30	15	309.3	251.2	30	20

Table A2. Matrix effects[a] of the tested pesticides.

	Cabbage	*Spinach*	*Potato*	*Eggplant*	*Orange*	*Apple*
2,4-D	89	81	95	86	97	90
2,4-DB	100	100	78	76	111	83
4-CPA	85	73	82	76	56	88
Acifluorfen	100	89	72	74	84	96
Azimsulfuron	90	94	86	86	78	100
Bensulfuron methyl	100	97	103	102	105	95
Bentazone	83	81	98	93	44	90
Bispyribac sodium	97	97	104	107	99	92
Bromoxynil	102	92	83	84	89	91
Bupirimate	95	93	99	97	93	82
Chlorimuron ethyl	96	94	100	91	105	87
Chlorsulfuron	90	92	92	92	102	90
Cinosulfuron	88	98	75	95	107	97
Clodinafop acid	89	93	83	79	94	83
Cloprop	97	72	112	105	106	88
Cloransulam methyl	100	99	106	102	116	101
Cyclanilide	107	94	81	93	93	96
Cyclosulfamuron	95	95	95	92	79	90
Dichlorprop	97	68	99	102	99	100

(Continued on next page)

Table A2. Matrix effects[a] of the tested pesticides. *(Continued)*

	Cabbage	*Spinach*	*Potato*	*Eggplant*	*Orange*	*Apple*
Diclosulam	95	96	101	100	87	86
Ethametsulfuron methyl	92	94	86	86	97	90
Ethoxysulfuron	90	93	87	89	83	85
Fenoprop	117	117	98	105	101	96
Flazasulfuron	86	79	81	81	102	79
Florasulam	95	95	94	93	112	92
Fluazifop	92	73	80	94	98	91
Flucarbazone sodium	99	100	105	94	100	95
Flucetosulfuron	109	103	111	102	89	101
Flumetsulam	99	97	100	96	92	97
Flusulfamide	81	95	99	88	69	81
Fomesafen	90	90	86	76	83	89
Foramsulfuron	93	94	78	85	120	86
Forchlorfenuron	95	61	98	45	80	77
Gibberellic acid	84	80	97	92	78	95
Halosulfuron methyl	83	91	90	72	94	78
Haloxyfop	92	92	98	96	76	89
Imazamethabenz methyl	91	96	84	88	85	94
Imazaquin	90	84	87	97	79	94
Imazosulfuron	88	82	69	90	97	82
Iodosulfuron methyl	96	103	110	96	105	79
Ioxynil	90	78	88	90	68	94
MCPA	98	102	99	90	96	96
Mecoprop	83	93	96	104	88	106
Mesosulfuron methyl	89	102	100	92	118	92
Mesotrione	94	95	95	95	111	96
Metosulam	95	102	105	92	110	93
Metsulfuron methyl	88	97	99	103	116	93
Naptalam	92	91	96	99	97	88
Nitenpyram	87	95	93	96	88	94
Oryzalin	97	97	84	79	80	92
Penoxsulam	100	105	104	103	108	98
Picloram	86	64	69	98	93	84
Primisulfuron methyl	94	105	120	75	94	86
Propoxycarbazone	101	99	93	98	116	99
Propyrisulfuron	83	92	86	95	85	83
Prosulfuron	87	96	106	91	100	79
Pyrasulfotole	92	89	82	59	86	89
Pyrazosulfuron ethyl	92	94	82	87	93	71
Pyrithiobac sodium	94	88	85	100	95	76
Quinclorac	87	92	99	89	93	88
Rimsulfuron	84	93	93	90	97	92
Sulfosulfuron	98	97	73	93	106	95
Tecloftalam	47	69	63	53	54	89
Thifensulfuron methyl	86	97	101	101	116	93
Tralkoxydim	90	90	78	98	86	84
Triasulfuron	97	94	94	95	104	91
Tribenuron methyl	92	75	95	52	67	54
Triclopyr	82	84	79	84	79	79
Trifloxysulfuron	91	97	79	88	90	85
Triflusulfuron methyl	110	95	104	105	99	91
Trinexapac	94	80	84	73	81	89
Trinexapac ethyl	92	92	88	79	96	91
Warfarin[b]	93	91	87	101	79	91

[a]Matrix effect (%) = peak area of matrix-matched standard/peak area of standard in solvent $\times 100$.
[b]fortified at 0.001 mg/kg.

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Multiresidue determination of pesticides in tea by gas chromatography-tandem mass spectrometry

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An efficient and reliable GC-MS/MS method for the multiresidue determination of pesticides in tea was developed by modifying the Japanese official multiresidue method. Sample preparation was carefully optimized for the efficient removal of coextracted matrix components. The optimal sample preparation procedure involved swelling of the sample in water; extraction with acetonitrile; removal of water by salting-out; and sequential cleanup by ODS, graphitized carbon black/primary secondary amine (GCB/PSA) and silica gel cartridges prior to GC-MS/MS analysis. The recoveries of 162 pesticides from fortified (at 0.01 mg kg^{-1}) green tea, oolong tea, black tea and matcha (powdered green tea) were mostly (95–98% of the tested pesticides) within the range of 70–120%, with relative standard deviations of <20%. Poor recovery of triazole pesticides was considered to be due to low recovery from the silica gel cartridges. The test solutions obtained by the modified method contained relatively small amounts of pigments, caffeine and other matrix components and were cleaner than those obtained by the original Japanese official multiresidue method. No interfering peaks were observed in the blank chromatograms, indicating the high selectivity of the modified method. The overall results suggest that the developed method is suitable for the quantitative analysis of GC-amenable pesticide residues in tea.

Keywords: GC-MS/MS, multiresidue method, pesticides, tea.

Introduction

Tea (*Camellia sinensis*) is one of the most widely consumed beverages in the world. Depending on its level of fermentation during the manufacturing process, tea can be classified into several groups, including green tea (unfermented), oolong tea (partially fermented) and black tea (fully fermented).[1] In Japan, a finely ground powdered green tea, known as matcha, is also consumed. Although tea is known to have a number of health benefits such as anticancer properties,[2,3] the consumption of tea can also be a potential source of exposure to pesticides used for tea cultivation control, resulting in long-term health effects. In addition, the concentration of pesticide residues in tea is generally high compared to other foods. As tea leaves have a large surface area per mass and the mass of tea leaves is reduced during the drying process, tea has high pesticide concentration.[4]

For these reasons, there is a demand for reliable multiresidue methods for analyzing pesticides present in tea. However, this is a challenging task, as tea leaves contain large amounts of complicated matrices such as pigments, polyphenols and caffeine, which contaminate LC-MS (/MS) and GC-MS(/MS) systems, thus interfering with analysis. Caffeine in particular is difficult to remove from tea extracts because it possesses similar chemical properties to a number of pesticides,[5] thus causing serious problems for GC-MS(/MS) analysis.

To date, there have been a number of reports on the multiresidue analysis of pesticide residues in tea using LC-MS(/MS) or GC-MS(/MS) techniques.[6–12] Recently, several modifications[13,14] of the QuEChERS (quick, easy, cheap, effective, rugged, safe) method, developed by Anastassiades et al.,[15] have been published. In Japan, an official "multiresidue method for agricultural chemicals" has been established (Fig. 1).[16] In addition, we previously reported the development of an LC-MS/MS method for the multiresidue analysis of pesticides in tea.[17] However, all these methods require frequent maintenance of the GC-MS/MS system, as they lack a cleanup step for the removal of caffeine from the system, resulting in a significant amount of caffeine in the later test solutions.

Thus, the aims of this study were as follows: (1) optimization of an efficient caffeine-removal cleanup step for the GC-MS/MS analysis of pesticide residues in tea; (2) development of a sensitive, selective and reliable analytical

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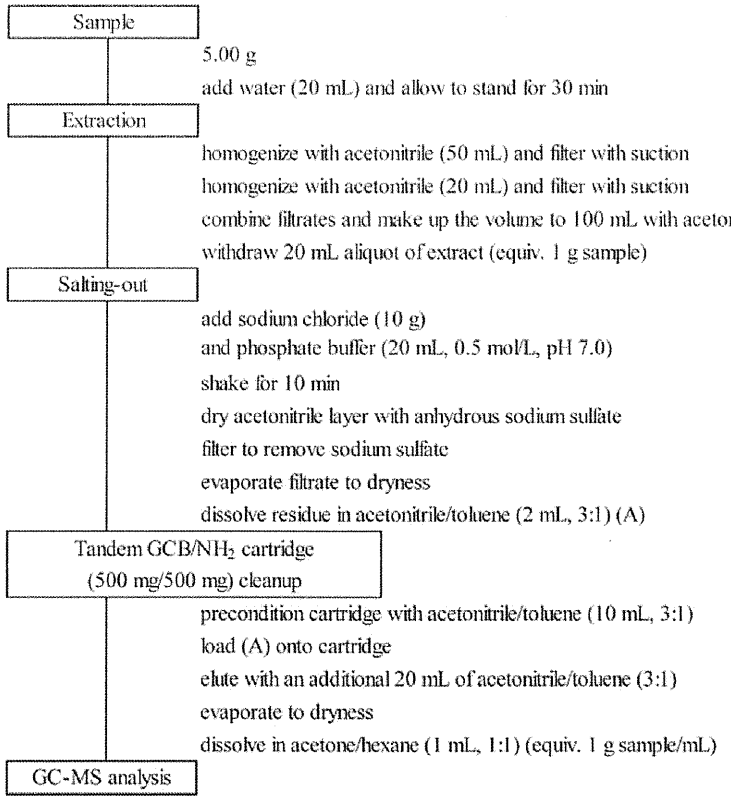

Fig. 1. Flow chart of sample preparation according to the Japanese official multiresidue method.

method for multiresidue determination of GC-amenable pesticides in tea by modifying the Japanese official multiresidue method; and (3) application of the proposed method to green tea, oolong tea, black tea and matcha.

Experimental

Reagents and materials

Solvents and reagents. Pesticide residue analysis-grade acetone, acetonitrile, hexane and toluene were obtained from Kanto Chemical (Tokyo, Japan). Diatomaceous earth (Celite, No. 545), pesticide residue analysis-grade sodium chloride, analytical-grade dipotassium hydrogen phosphate and analytical-grade potassium dihydrogen phosphate were purchased from Wako Pure Chemical Industries (Osaka, Japan). The water used to prepare the test solutions was purified using an NZJ-2DSYW distillation apparatus (Fujiwara Scientific, Tokyo, Japan).

Analytical standards. Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Riedel-de Haën (Seelze, Germany), Sigma-Aldrich (St. Louis, MO, USA), Hayashi Pure Chemical (Osaka, Japan), Wako Pure Chemical Industries, and the Kanto Chemical Co. Individual stock standard solutions (1,000 mg L^{-1}) were prepared in hexane, or in a mixture of hexane and acetone, depending on the solubility of each pesticide. Working standard solutions were prepared by mixing the stock standard solutions, and diluting with acetone and hexane as required.

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

Phosphate buffer. Phosphate buffer (0.5 mol L^{-1}, pH 7.0) was prepared by dissolving dipotassium hydrogen phosphate (52.7 g) and potassium dihydrogen phosphate (30.2 g) in water (500 mL). The pH was then adjusted to

pH 7.0 by the addition of either 1 mol L^{-1} sodium hydroxide or 1 mol L^{-1} hydrochloric acid. The final volume of the solution was made up to 1 L with water.

Food samples. Green tea leaves, oolong tea leaves, black tea leaves and matcha (powdered green tea), were purchased from a market in Tokyo (Japan). Green tea leaves, oolong tea leaves and black tea leaves were ground into small particles using a centrifugal mill and passed through a 425 μm pore standard sieve.

Apparatus

GC-MS/MS. A Trace 1310 Gas Chromatograph (ThermoFisher Scientific, MA, USA) coupled to a TSQ 8000 Mass Spectrometer (ThermoFisher Scientific), equipped with a TriPlus RSH Autosampler (ThermoFisher Scientific) was used under the following operating conditions: column, DB-5ms column (30 m length, 0.25 mm id, 0.25 μm film thickness; Agilent) equipped with a guard column (2 m length, 0.25 mm id; Agilent); column temperature, 50 °C (1 min), increased to 125 °C at 25 °C min^{-1}, then increased to 300 °C at 10 °C min^{-1} and held for 8.5 min; carrier gas, helium; flow rate, 1 mL min^{-1}; injection volume, 2 μL; ionization mode, electron impact (EI); ion source temperature, 260 °C; injection port temperature, 260 °C; transfer line temperature, 280 °C. The selected reaction monitoring (SRM) transitions and retention times are summarized in Table A1.

Laboratory apparatus. A homogenizer (Polytron PT 10–35 GT; Kinematica, Lucerne, Switzerland), centrifugal mill (Ultra Centrifugal Mill ZM 200; Retsch, Haan, Germany), rotary evaporator (N-1000/NVC-2100; Tokyo Rikakikai, Tokyo, Japan), electric shaker (SR-2w; Taitec, Saitama, Japan) and centrifuge (Centrifuge 8100; Kubota, Tokyo, Japan) were used in the studies as described.

Sample preparation

The desired sample (5.00 g) of tea was weighed in a 350 mL glass tube and was allowed to stand for 30 min after the addition of water (20 mL). After this time, the mixture was extracted with acetonitrile (50 mL) using a homogenizer. The homogenate was filtered under reduced pressure through Celite filter aid, and the residue was re-homogenized with acetonitrile (20 mL) and filtered once more under reduced pressure. The volume of the combined extract was made up to 100 mL with acetonitrile. An aliquot of the extract (20 mL) was added to a 50 mL PTFE centrifuge tube containing sodium chloride (10 g) and phosphate buffer (20 mL, 0.5 mol L^{-1}, pH 7.0). The mixture was shaken vigorously for 10 min and centrifuged at 3,000 rpm for 5 min. The resulting acetonitrile layer was loaded onto a preconditioned (10 mL acetonitrile) ODS cartridge and eluted with an additional portion of acetonitrile (5 mL). The combined eluate was concentrated to approximately 1 mL using a rotary evaporator (<40°C) and evaporated to dryness under a stream of nitrogen. The residue was subsequently redissolved in acetonitrile (3 mL) assisted by ultrasonication and an aliquot of toluene (1 mL) was added. The resulting solution was loaded onto a GCB/PSA cartridge and eluted with a mixture of acetonitrile/toluene (18 mL, 3:1). The combined eluate was concentrated to approximately 1 mL using a rotary evaporator (<40°C), evaporated to dryness under a stream of nitrogen and the residue was redissolved in an acetone/hexane mixture (2 mL, 3:7). The resulting solution was loaded onto a silica gel cartridge and eluted with a mixture of acetone/hexane (13 mL, 3:7). The combined eluate was concentrated to approximately 1 mL using a rotary evaporator (<40°C), evaporated to dryness under a stream of nitrogen, and finally, the residue was redissolved in an acetone/hexane mixture (1 mL, 1:1).

Recovery test

The recovery tests were performed for green tea, oolong tea, black tea and matcha (powdered green tea), at 0.01 mg kg^{-1} spike level, with each test being replicated five times for all matrices. An aliquot (0.5 mL) of the working mixed standard solution of 162 pesticides was added to each sample and the mixture was allowed to stand for 30 min before extraction. The recoveries of the individual pesticides were calculated on the basis of the peak areas of each pesticide, and they were quantified using matrix-matched calibration (25%, 50%, 75%, 100%, 125% and 150%).

Preparation of matrix-matched standards

Matrix-matched standards were prepared as follows. Extracts of blank samples (100 μL) were evaporated to dryness under a stream of nitrogen, and the residue was redissolved in the standard acetone/hexane solution (100 μL, 1:1).

Results and discussion

Optimization of sample preparation

Extraction and salting-out. As acetonitrile is the most commonly used extraction solvent for the multiresidue method for pesticides and it is also used in the Japanese official multiresidue method (Fig. 1), it was chosen as the extraction solvent for our studies. The extraction efficiency of the incurred pesticide residues obtained from tea samples was compared by Cajka et al., both with and without matrix swelling prior to extraction with acetonitrile.[6] They demonstrated that the addition of water to the sample matrix is a key factor for achieving the maximum

extraction yield. Therefore, in this study, we mixed water (20 mL) with tea leaves (5.0 g) and allowed them to swell for 30 min prior to extraction with acetonitrile. Kanrar et al. assessed the extractability of both polar and nonpolar pesticide residues by shaking, blending and vortexing prepared tea and spent leaves and comparing the results obtained.[7] Their studies revealed that blending gave better recoveries for the majority of pesticides, compared to shaking or vortex-based methods. Thus, in this study, we adopted a homogenizing procedure using acetonitrile for extraction, as in the Japanese method. In addition, as high pesticides recoveries were obtained in the salting-out procedure conducted with the aid of sodium chloride and phosphate buffer, and both water and polar matrix components were efficiently removed from the extract, we also chose to adopt a salting-out step, as in the Japanese official multiresidue method.

Cleanup. In order to remove matrix components of low polarity, cleanup using an ODS cartridge was investigated. The acetonitrile layer obtained after salting-out was loaded directly onto an ODS cartridge and eluted with an additional 5 mL of acetonitrile. Although the yellow pigments from the tea extract eluted from the ODS cartridge, the majority of green pigments (chlorophylls) and other matrix components of low polarity remained on the cartridge. All pesticides examined in the study eluted from the

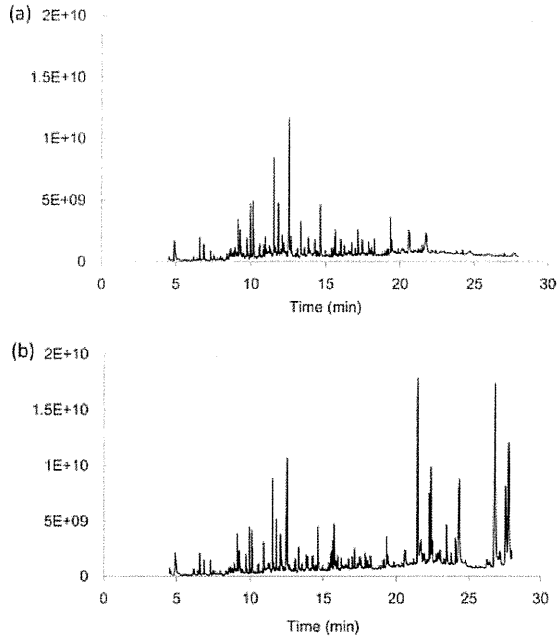

Fig. 2. Total ion current (TIC) chromatograms of the blank green tea sample, (a) with ODS cartridge cleanup and (b) without ODS cartridge cleanup.

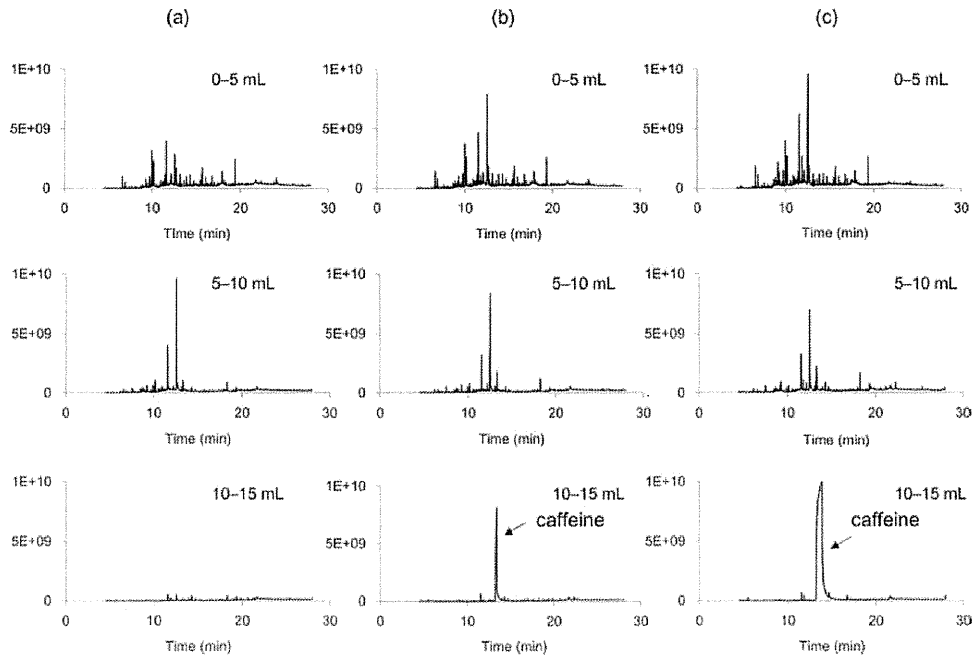

Fig. 3. Total ion current (TIC) chromatograms of a blank green tea sample after cleanup with a silica gel cartridge, eluting with (a) acetone/hexane (3:7), (b) acetone/hexane (4:6) and (c) acetone/hexaen (5:5). Elution volume: upper 0–5 mL; middle 5–10 mL; lower 10–15 mL.

cartridge in high recoveries. Figure 2 shows the total ion current (TIC) chromatograms of green tea extract, both with and without ODS cartridge cleanup. It can be seen from Figure 2 that the number of coextracted matrix peaks (retention time >21 min) was smaller in the TIC chromatogram of the sample that had been subjected to ODS cartridge cleanup. Consequently, cleanup using an ODS cartridge was included in our method after the salting-out step, unlike in the Japanese official multiresidue method.

As shown in Figure 1, the Japanese official multiresidue method involves a tandem GCB/aminopropyl silanized silica gel (NH_2) cartridge cleanup. However, as PSA is a stronger anion-exchange sorbent than the NH_2-based sorbent, and can retain acidic coextracted matrix components more efficiently, we selected the tandem GCB/PSA cartridge for use in this study.

In the cleanup procedure of the Japanese official multiresidue method (Fig. 1), the residue obtained from the salting-out step is dissolved in an aliquot of acetonitrile/toluene (2 mL, 3:1) to load onto the tandem GCB/NH_2 cartridge. However, because tea contains a large amount of matrix components, the residue obtained from the ODS cartridge cleanup was insoluble in the acetonitrile/toluene mixture. This was particularly the case for fermented black tea, most likely due to the presence of high-molecular-weight polyphenols, which could not be well partitioned in the aqueous phase during the salting-out step. In this study, we therefore chose to dissolve the residue in a small amount of a relatively high polar

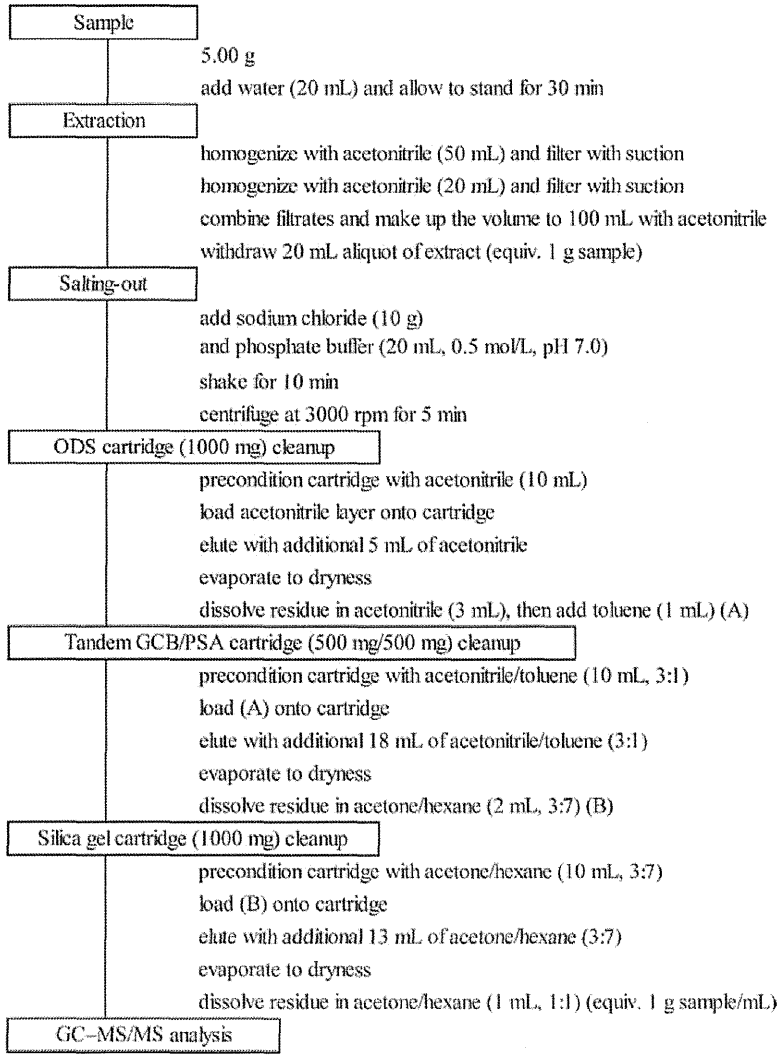

Fig. 4. Flow chart of sample preparation according to the modified multiresidue method.

solvent, acetonitrile (3 mL), with the aid of ultrasonication. The relatively apolar toluene (1 mL) was then added to the sample prior to loading onto the tandem GCB/PSA cartridge. While poor recoveries were obtained for coumaphos (50%), diflufenican (58%), pyrazophos (64%) and tecnazene (69%) using the standard mixture in pure solvent, all other pesticides were obtained in satisfactory recoveries from this system by elution with acetonitrile/toluene (3:1).

Although the tandem GCB/PSA cartridge cleanup effectively removed acidic matrix components together with other pigments from the samples, a large interfering peak with a retention time of 13.4 min was observed in the TIC chromatograms of tea extracts after the tandem GCB/PSA cartridge cleanup. This peak is due to caffeine, which is present in large quantities (1–4%)[1] in tea extracts. The presence of such a significant quantity of caffeine in the final test solution for GC-MS/MS analyses would be troublesome, since this can affect the GC-MS/MS performance and cause considerable matrix effects. In order to avoid the requirement for frequent maintenance of GC-MS/MS systems, the introduction of a further cleanup step was investigated to reduce the caffeine content prior to GC-MS/MS analysis. As caffeine is a relatively polar compound compared to the pesticides being examined here, a silica gel cartridge was chosen for the separation of pesticides and caffeine, and the elution solvent was optimized by varying the proportion of acetone to hexane.

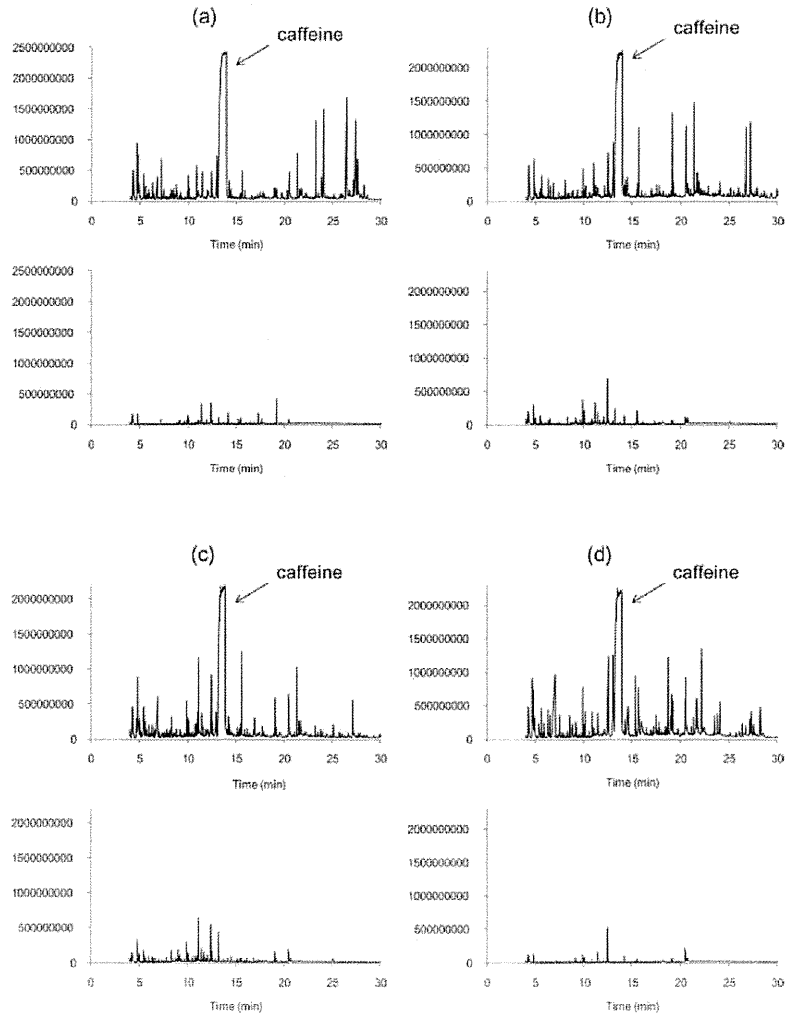

Fig. 5. Total ion current (TIC) chromatograms of (a) green tea, (b) oolong tea, (c) black tea and (d) matcha samples prepared by the Japanese official multiresidue method (upper) and the modified multiresidue method (lower).

Table 1. Recovery of pesticides from green tea, oolong tea, black tea and matcha (powdered green tea).

	Green tea		*Oolong tea*		*Black tea*		*Matcha (Powdered green tea)*	
Pesticide	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*
Acetochlor	93	2	91	4	93	4	83	4
Acrinathrin	85	5	83	2	83	5	77	3
Alachlor	99	2	92	3	91	3	83	1
Aldrin	80	4	84	5	82	8	71	2
Ametryn	96	7	89	5	87	3	80	2
Anilofos	92	5	90	5	94	3	81	7
Aramite	97	6	93	2	93	7	84	3
Atrazine	100	7	92	2	90	8	82	3
Azinphos methyl	94	3	88	3	88	3	80	3
Azoxystrobin	93	4	90	5	90	3	82	11
Benalaxyl	96	2	96	1	89	3	85	4
Benfluralin	86	6	89	2	93	3	82	15
Benfuresate	97	2	93	1	92	1	83	6
Benoxacor	96	2	90	2	91	3	82	4
α-BHC	92	4	89	3	90	6	79	4
β-BHC	100	5	88	2	88	4	81	4
γ-BHC	95	5	91	3	89	5	80	2
δ-BHC	98	6	89	3	90	4	78	3
Bifenox	93	5	91	5	82	4	83	5
Bifenthrin	93	4	91	1	83	5	79	2
Bitertanol	34	15	60	14	70	16	17	4
Bromobutide	95	1	91	3	90	5	95	7
Bromophos	94	6	88	4	84	6	82	13
Bromophos ethyl	93	8	89	7	86	5	79	16
Bromopropylate	92	3	90	3	84	4	84	11
Bupirimate	94	5	103	5	89	10	89	7
Butachlor	92	2	94	3	87	5	80	3
Butafenacil	95	2	91	1	90	6	82	9
Butamifos	90	2	92	5	92	2	81	8
Cadusafos	95	1	91	2	88	5	88	2
Cafenstrole	88	3	88	2	88	5	79	4
Carfentrazone ethyl	96	3	92	2	95	3	93	5
Chlorbenside	92	4	84	2	85	1	73	7
Chlordane (*cis*)	90	5	84	4	86	8	82	13
Chlordane (*trans*)	89	6	81	2	82	9	82	17
Chlorfenson	97	6	92	3	92	5	84	3
Chlorfenvinphos	96	4	89	4	87	4	88	12
Chlorobenzilate	93	3	91	3	90	2	79	6
Chlorpropham	95	2	90	1	92	4	83	4
Chlorpyrifos	91	5	92	6	86	3	83	13
Chlorpyrifos methyl	92	3	89	5	92	4	81	12
Chlorthal dimethyl	93	10	87	5	87	3	84	14
Clomazone	96	6	90	2	90	4	83	3
Clomeprop	93	3	93	2	85	4	85	9
Coumaphos	98	1	92	7	87	4	83	4
Cyanazine	99	3	90	5	83	4	78	6
Cyflufenamid	94	16	87	9	91	12	78	10
Cyfluthrin	93	3	90	2	91	4	83	3
Cyhalothrin	93	2	95	3	88	3	79	5
Cyproconazole	36	7	44	9	56	17	24	3
Cyprodinil	92	1	89	2	79	8	79	7
Deltamethrin	86	4	87	3	88	3	79	3
Di-allate	88	2	89	1	87	3	77	5

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Table 1. Recovery of pesticides from green tea, oolong tea, black tea and matcha (powdered green tea). *(Continued)*

	Green tea		*Oolong tea*		*Black tea*		*Matcha (Powdered green tea)*	
Pesticide	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*
Diazinon	95	5	101	3	92	8	88	3
Dichloran	90	5	89	2	87	4	80	4
Dieldrin	99	11	84	18	91	11	73	6
Diflufenican	95	5	90	8	90	4	86	9
Dimethametryn	96	3	93	1	88	5	83	3
Dimethenamid	96	3	93	1	88	3	82	5
Dimethoate	77	3	73	8	72	10	71	2
Dimethylvinphos (*E*)	95	4	90	3	90	4	83	4
Dimethylvinphos (*Z*)	93	2	91	2	89	5	80	7
Disulfoton	85	6	56	9	71	5	78	5
Dithiopyr	94	3	88	10	91	6	83	10
Edifenphos	90	4	90	7	87	3	80	5
α-Endosulfan	94	8	85	10	88	9	80	6
β-Endosulfan	100	6	90	4	93	12	81	5
Endosulfan sulfate	98	13	95	2	89	6	79	4
Endrin	92	6	86	10	93	7	74	1
EPN	91	4	89	3	82	3	80	3
Esprocarb	95	2	91	2	92	6	83	6
Ethion	93	1	90	1	91	2	79	6
Ethoprophos	96	2	89	2	90	4	83	2
Etofenprox	93	5	82	3	88	5	79	3
Etoxazole	93	2	91	4	89	9	88	12
Fenarimol	112	7	92	2	89	4	80	3
Fenchlorphos	91	5	93	3	95	3	84	13
Fenitrothion	91	8	89	6	92	2	81	9
Fenoxanil	97	6	93	5	92	3	81	9
Fenpropimorph	76	7	69	9	66	12	59	4
Fenvalerate	90	4	90	1	88	4	80	3
Fipronil	92	2	94	7	87	5	81	4
Flamprop methyl	98	1	93	3	89	4	82	7
Flucythrinate	95	3	90	3	91	3	81	4
Fludioxonil	91	1	91	7	92	2	80	10
Fluquinconazole	95	1	91	4	85	4	81	7
Flutolanil	97	4	91	3	91	4	84	7
Fosthiazate	92	5	89	7	86	4	86	5
Fthalide	95	3	83	7	80	4	78	8
Indoxacarb	100	8	90	9	90	4	84	3
Iprobenfos	93	4	91	4	91	3	82	1
Isazophos	99	6	90	7	89	5	81	5
Isofenphos	96	2	92	2	92	4	83	6
Isofenphos oxon	91	6	92	6	88	5	84	7
Isoprocarb	86	3	89	2	89	1	81	3
Isoprothiolane	98	3	94	5	92	8	89	9
Isoxadifen ethyl	96	6	90	4	90	4	81	8
Isoxathion	108	3	92	8	84	6	88	6
Kresoxim methyl	99	1	90	3	91	4	82	6
Lenacil	89	5	84	5	83	4	76	3
Malathion	95	2	91	7	91	5	84	2
Mefenacet	98	4	90	2	89	5	81	2
Mefenpyr diethyl	96	2	92	4	91	1	82	6
Mepronil	92	4	91	5	92	2	85	10
Metalaxyl	91	1	87	7	84	4	78	6
Methidathion	91	3	91	2	91	5	83	6

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Table 1. Recovery of pesticides from green tea, oolong tea, black tea and matcha (powdered green tea). *(Continued)*

	Green tea		*Oolong tea*		*Black tea*		*Matcha (Powdered green tea)*	
Pesticide	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*	*Recovery (%)*	*RSD%*
Methoxychlor	106	4	91	9	85	4	84	4
Metolachlor	92	2	89	5	90	3	83	2
Myclobutanil	5	13	10	89	22	70	4	0
Oxadiazon	96	6	88	8	88	8	85	6
Oxadixyl	86	5	83	3	86	3	74	7
Paclobutrazol	88	4	88	3	88	3	77	6
Parathion	90	2	92	12	86	8	83	8
Parathion methyl	94	3	92	9	91	5	84	8
Penconazole	32	24	67	7	72	12	13	5
Pendimethalin	91	4	89	4	86	4	76	6
Permethrin	93	6	88	6	88	5	81	2
Phenothrin	100	8	88	9	82	11	81	3
Phenthoate	88	3	90	2	93	3	80	4
Phosalone	94	3	88	4	89	2	82	2
Phosmet	91	5	83	5	77	5	76	3
Piperonyl butoxide	95	4	85	7	90	2	81	4
Procymidone	97	3	94	2	91	8	85	11
Profenofos	86	2	91	10	92	6	88	11
Prometryn	91	4	88	4	88	4	84	3
Propiconazole	91	3	90	8	86	6	68	11
Propoxur	85	3	86	5	86	4	81	2
Propyzamide	98	4	89	3	93	5	83	3
Prothiofos	89	6	87	6	86	2	82	12
Pyraflufen ethyl	93	8	93	12	88	3	79	13
Pyrazophos	94	3	90	6	90	2	80	5
Pyributicarb	92	4	87	9	89	7	81	1
Pyridaben	95	3	89	4	88	3	78	11
Pyridafenthion	91	3	90	2	88	7	78	7
Pyrifenox (*E*)	87	5	83	4	78	7	77	5
Pyrifenox (*Z*)	93	3	83	4	83	6	81	5
Pyrimethanil	95	7	86	4	84	4	79	3
Pyriminobac methyl (*E*)	96	2	93	1	90	1	84	5
Pyriminobac methyl (*Z*)	97	2	92	3	87	3	84	7
Pyriproxyfen	91	1	89	3	89	5	80	3
Quinoxyfen	92	4	85	3	83	4	74	8
Quintozene	85	3	84	10	76	5	77	8
Simeconazole	12	14	31	41	51	33	1	56
Tebufenpyrad	97	2	92	8	93	4	89	8
Tecnazene	80	2	82	4	81	1	76	7
Tefluthrin	95	5	86	4	89	5	81	5
Terbufos	90	2	84	3	85	5	79	4
Tetrachlorvinphos	94	3	88	2	85	4	80	4
Tetradifon	97	6	89	6	86	6	81	8
Thenylchlor	93	3	92	3	89	4	87	4
Thiobencarb	97	3	86	7	93	6	77	9
Tolclofos methyl	94	4	90	2	90	3	78	9
Tolfenpyrad	92	3	91	7	87	3	79	3
Triadimefon	89	9	95	5	90	5	83	10
Triadimenol	77	5	81	5	87	2	51	18
Tri-allate	91	2	89	4	87	2	77	8
Triazophos	92	6	94	4	92	3	80	9
Tribuphos	100	3	89	8	89	6	82	6
Trifloxystrobin	88	10	90	9	94	10	83	6

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