Multi-residue Analysis of 18 Pesticides in Fresh Fruits, Vegetables and Rice by Supercritical Fluid Extraction and Liquid Chromatography-Electrospray Ionization Mass Spectrometry

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(Received August 20, 2001; Accepted December 26, 2001)

A multi-residue screening method was developed for the determination of 18 pesticides in fresh fruits, vegetables and unpolished rice by supercritical fluid extraction (SFE), cleaned up with cartridge columns, and liquid chromatography-mass spectrometry with electrospray (LC/MS (ESI)). Comparing with our previous method, the number of fraction at purification was reduced to one fraction in order to reduce the preparation time, and detection limit and recovery value of almost pesticides were improved. The detection limits of the 18 pesticides were 0.04–148 ng/g in sample. Furthermore, in case of crops including many interfering peaks by UV detection, using a LC-MS (SIM) significantly improved the quantitative and qualitative analyses with less interfering peaks than UV detection.

Key words —— pesticide, supercritical fluid extraction, liquid chromatography-mass spectrometry

INTRODUCTION

The multi-residue analysis is useful for a screening examination of pesticides, because the official methods for pesticide analysis based on the Japanese Food Sanitation Law are mostly individual analysis. We have previously reported that the screening method for 27 pesticides in fruits and vegetables extracted with supercritical fluid extraction (SFE), cleaned up with cartridge columns and then measured by HPLC with a UV detector.[1] However, the chromatograms of some crop samples have many interference peaks. Therefore, the target pesticides peaks need to be separated from the interference peaks by a LC column or other specific detectors, such as a fluorescence detector, an electron conductivity detector, and a mass spectrometer. Furthermore, there are some problems of this method including time consuming step such as dividing the eluate into three fractions to reduce the interfering substances, and the samples of some crops still have many interfering substances.

Recently, liquid chromatography-mass spectrometry (LC-MS) has been applied to biological samples, such as vitamins, pigments, medicine, endocrine-disruptors, food additives, antibiotics and so on.[2] It is well known that LC-MS detection has less interfering peaks than UV detection, because LC-MS can detect a specific *m/z* of a target compound. Concerning pesticide residue analysis, only a few attempts were made for multi-residue analyses by LC-MS except for the *N*-methylcarbamates,[3,4] and the almost pesticide residue analyses by LC-MS were reported as individual analyses.

In this report, the multi-residue screening method for pesticides regulated in Japan is developed using SFE and LC-MS. The aim of this study was to establish a more simplified method based on previous our method for pesticide residues except for *N*-methylcarbamates in vegetables, fruits and unpolished rice using LC-MS, and to mention the problems on the LC-MS determination.

MATERIALS AND METHODS

Apparatus ——

SFE: Super-201 (JASCO, Hachioji, Japan). Extraction conditions: extraction fluid, CO_2; pressure, 300 kg/cm^2; extraction temperature, 40°C; flow rate of CO_2, 4.9 ml/min; flow rate of acetone modifying CO_2, 0.1 ml/min; extraction time, 40 min; extraction vessel volume, 10 ml. Trapping conditions of extract: nozzle temperature, 75°C; trap packing of column, layered Extrelut® NT (Merk kgaA Dalmstalt, Germany) 1 g over BondElut® C18 (Varian, Harbar City CA, U.S.A.) 1 g into a glass tube (150 × 14 mm i.d.).

LC-MS System: The LC-MS system included a

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Shimadzu (Kyoto, Japan) LC-MS-QP8000 liquid chromatograph/mass spectrometer. LC separations were performed with a Wakosil-II 3C18HG column (150 mm × 2.0 mm i.d., 3 μm particle diameter, Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). The flow rate was 0.2 ml/min with an oven temperature of 50°C and an injection volume of 5 μl. Solvent A and solvent B were water and acetonitrile respectively. The gradient elution condition was initially A-B (7 : 3), programming to 100% B over 18 min, and holding 100% B for 8 min. The column equilibrated under the initial condition for 7 min prior to the next injection.

Reagent and Pesticide standards ——

Reagent: Pesticide residue analysis grade acetonitrile, acetone, ether, *n*-hexane and diatomaceous earth Celite 545 were purchased from Wako Pure Chemical Industries, Co., Ltd. Liquid CO_2 (99.99% pure) was from Kinki Sanso Co., Ltd. (Sasayama, Japan). A water-absorbent polymer Arasorb S-310 was obtained from Arakawa Chemical Industries, Co., Ltd. (Osaka, Japan). Bond Elut® C_{18}; PSA (500 mg: Varian, Harbor City, CA, U.S.A.) and Sep-Pak® Florisil (690 mg: Waters, Massachusetts, U.S.A.) were used for purification. All other reagents used were of the highest grade commercially available.

Pesticide Standards: Pesticides standards were purchased from Wako Pure Chemical Industries, Co., Ltd. Each stock solution was prepared at 1.0 mg/ml in acetonitrile. Standard working solutions of various concentrations were prepared by appropriate dilution of acetonitrile.

Sample Preparation and Analysis ——

Extraction: Commercially purchased cucumber, potato, apple, radish, banana and unpolished rice served as the blank or spiked sample. Five grams of the homogenized sample, 1 g of Celite and 1 g of Arasorb S-310 polymer were mixed well with a glass rod. To completely granulate the mixture, a bit of Celite was added to it and mixed well. Since cereals are less water content, Celite and Arasorb were unnecessary at the assay of rice. Thereafter, the mixture was packed into the extraction vessels. For the fortified samples, the pesticide mixture was added to the sample in the vessel. The pesticides which were extracted by SFE were trapped with the column layered with Extrelut® NT and Bond Elut® C_{18}. Powder of unpolished rice was extracted by SFE without the water-absorbent polymer,[5] and the extracts from SFE were trapped in the same manner (Extrelut® NT and Bond Elut® C_{18}).

Purification: The column trapping the pesticides was removed from the SFE instrument, the pesticides were eluted with 40 ml of acetonitrile, and the eluate was evaporated to dryness. The residue was dissolved in 2 ml of *n*-hexane, and then applied to the Bond Elut® PSA over Sep-Pak® Florisil columns connected in tandem.

After rinse with *n*-hexane, the pesticides were sequentially eluted from these cartridges with 50 ml of 50% acetone/*n*-hexane. The eluate was evaporated to dryness. The residue was dissolved and filled up to exactly 1 ml with acetonitrile, and the sample was measured by electro spray ionization (ESI) mode of LC-MS QP8000.

Optimization of LC-MS Parameters: Eighteen pesticides were separated by reverse phase chromatography using water-acetonitrile gradient. To determine the proper voltage condition of LC-MS, the target pesticides were measured by both negative and positive mode with various voltages by a flow injection. The LC-MS conditions were shown in Table 1.

RESULTS AND DISCUSSION

LC-MS Condition

The LC condition during the LC-MS measurement was the same as in our previous report.[1] The ionization on LC-MS was performed by ESI, the moderate ionization way. Since almost mass spectra of target pesticides by LC-MS (ESI) were simple

Table 1. Condition of Method A, B and C at LC-MS Detection

Method	R.T. (min)	CDL (V)*	DEF (V)**	Monitor ion (m/z)				
A	0–25	−30	50	339	289	297	346	306
B	0–10.5	−25	45	222	334	342		269
	10.5–11.75	−35	45	269				
	11.75–25	−35	55	297	299	353		403
C	0–25	20	−50	309	459	487		538

*CDL is an abbreviation for Curred Desolvation Line. **DEF is an abbreviation for deflector.

and have one peak (molecular ion), both qualitative and quantitative analyses could be done by selected ion monitoring (SIM). We tried to apply the 27 pesticides, examined by LC (UV) analysis in our previous report, to LC-MS. The result of the experiment was that the 18 pesticides could be measured by LC-MS and the proper determination mode in polarity (negative or positive) was that pesticides Nos. 1–14 were measured by positive mode and Nos. 15–18 were done by negative mode (see Table 1). The QP-8000 could not change the polarity of the during data acquisition, therefore they were individually measured by each polarity. Every pesticide had different suitable voltage value in the MS condition, however, it is difficult to measure the pesticides using the plural voltage at the same time. The reason for this was that each peak was not perfectly separated and the each pesticide of overlapped peaks had different suitable voltage value, so that such pesticides could not measured by same voltage conditions. Therefore, two methods of positive mode and one method of negative mode were utilized. Table 2 shows the target *m/z* values and limit of detection in sample by LC-MS. The LC-MS chromatograms of the standard pesticides are shown in Fig. 1.

The amount of the pesticides, Nos. 3, 5, 6, 9, 10, 12, 13 and 14, were lower than the amount in our previous report, because these were high sensitivity in the LC-MS measurement. Especially, about imazalil in Table 2, LC-MS had approximately a 20 times greater sensitivity than HPLC (UV).

Cleanup

Pesticides trapped in the column were eluted with 40 ml of acetonitrile and the eluate was purified using Sep-Pak® Florisil and Bond Elut® PSA column. In our previous method, carrot, onion and orange included many interfering substances, and spiked pesticides in such crops could not be determined by HPLC (UV). In MS detection, since a specific *m/z* of the target compound is set, the pesticides can be detected even if there are overlapped interfering peaks.

Recovery Test from Agricultural Products

In this study, rice was also added as target agricultural products. The recoveries of 18 pesticides added to 2.5 μg (0.1 μg/g equivalency) or 12.5 μg (0.5 μg/g equivalency) in six agricultural products are shown in Table 3. The recoveries were greater than approximately 60%. Especially, the recoveries of imazalil were considerably improved compared with our previous study. The recovery ranges of almost target pesticides were within 60–140% of an acceptable range as screening method except for buprofezin in the all crops, myclobutanil in cucumber and banana, tebufenozide in rice and fenpyroximate in cucumber and rice. The range of standard deviations (SD) of almost pesticides was 10–15%, however some of them were exceeded

Table 2. Limits of Detection by LC/MS (SIM) and Monitor Ion (m/z)

Method	No.	Target pesticide	Limits of detection (ng/g)	Monitor ion (m/z)
A	1	Inabenfide	3.6	339
	2	Myclobutanil	24	289
	3	Imazalil	0.04	297
	4	Triflumizole	24	346
	5	Buprofezin	0.04	306
B	6	Methabenzthiazuron	32	222
	7	Furametpyr	12	334
	8	Benfuresate	28	342
	9	Dymron	0.08	269
	10	Iprodione	0.12	297 299
	11	Tebufenozide	112	353
	12	Pyrazoxyfen	0.08	403
	13	Pencycuron	0.04	329
	14	Fenpyroximate	0.04	422
C	15	Diflubenzuron	148	309
	16	Hexaflumuron	0.04	459
	17	Flufenoxuron	0.4	487
	18	Chlorfluazuron	16	538

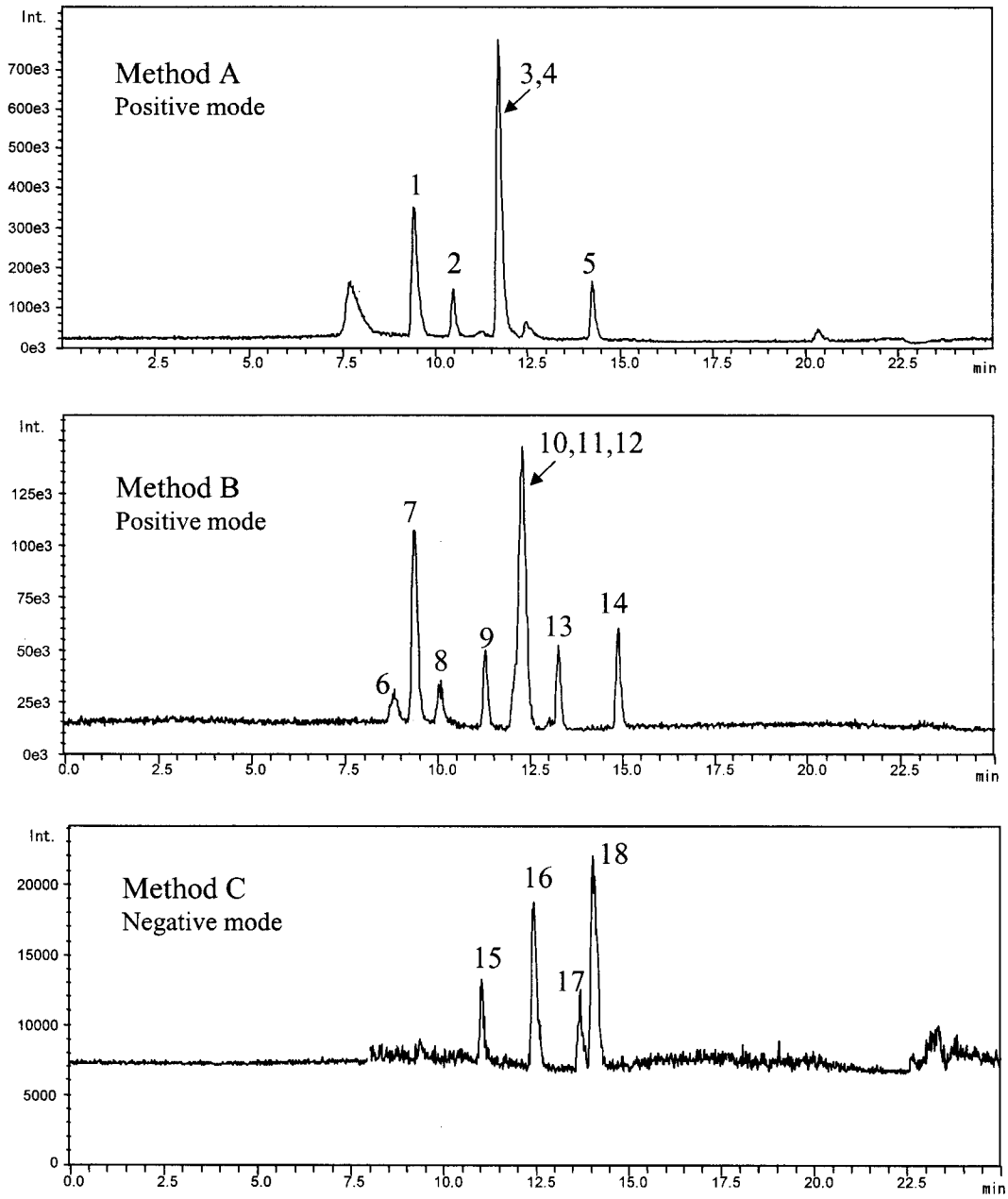

Fig. 1. MIC Chromatograms of Pesticides Standard Mixture*

1. Inabenfide, 2. Myclobutanil, 3. Imazalil, 4. Triflumizole, 5. Buprofezin, 6. Methabenzthiazuron, 7. Furametpyr, 8. Benfuresate, 9. Dymron, 10. Iprodione, 11. Tebufenozide, 12. Pyrazoxyfen, 13. Pencycuron, 14. Fenpyroximate, 15. Diflubenzuron, 16. Hexaflumuron, 17. Flufenoxuron, 18. Chlorfluazuron. *Concentration level are 12.5 ng (Nos. 1, 2, 4, 7, 8, 11) and 2.5 ng (the others Nos).

20%. Generally, SD values of LC-MS were greater than that of UV. It was postulated that the phenomenon was resulted from variation of the ionization coefficient, which was influenced by interfering substances from crops or maintenance condition of MS instrument.[6)]

Figure 2 shows typical MIC (Mixed Ion Chromatogram) of apple and rice. The UV chromatograms of these samples show interfering peaks close to the retention times of the pesticides. In contrast to UV detector, LC-MS (SIM) could detect and measure almost target pesticides.

In conclusions, in this report, the multi-residue screening method for pesticides regulated in Japan is developed using SFE and LC-MS. The recovery ranges of almost target pesticides were within 60–140% of an acceptable range as screening method except for some cases. The limits of detection of the target pesticides by developed method were from 0.04 ng/g for imazalil to 148 ng/g for diflubenzuron

Table 3. Recoveries of 18 Pesticides Added to Agricultural Products by LC/MS (SIM) Method

No.	Pesticides	Recovery (%) mean ± S.D. ($n = 3$–5)					
		Potato	Radish	Apple	Cucumber	Banana	Rice
1	Inabenfide*	73.5±13.6	71.2± 9.6	87.0± 5.3	74.2±10.9	78.6± 7.0	85.5± 9.6
2	Myclobutanil*	77.9±18.4	63.0±12.8	70.5± 8.4	58.6± 8.3	55.7± 9.5	64.0± 9.5
3	Imazalil	79.2±11.5	65.1± 8.2	83.6±11.5	61.9± 8.8	66.2±16.3	71.7±10.8
4	Triflumizole*	62.4±16.0	92.4± 9.8	77.1± 8.5	104.3± 9.1	88.2± 9.4	84.7±11.0
5	Buprofezin	50.1±15.9	55.7± 7.7	48.3± 5.3	29.4±14.9	38.4±11.0	36.2±10.9
6	Methabenzthiazuron	75.0± 1.7	86.5± 1.4	82.2± 1.6	87.8±15.4	74.4±10.4	81.8± 6.9
7	Furametpyr*	73.1± 8.2	72.4± 1.5	87.6± 4.6	87.9± 4.0	78.2± 7.2	83.2±14.6
8	Benfuresate*	75.4± 7.2	76.0± 6.3	86.3± 1.3	96.7± 8.4	84.8± 4.0	78.6± 4.4
9	Dymron	81.9± 6.4	79.8± 8.1	95.7± 6.1	91.4± 3.1	76.9± 9.0	72.3± 4.8
10	Iprodione	69.0±15.2	79.7±11.9	93.0± 9.9	91.4± 9.5	80.1± 7.3	90.4± 6.2
11	Tebufenozide*	83.4±10.2	83.9±11.5	85.5± 7.5	71.4± 8.9	71.1±15.9	48.9±27.9
12	Pyrazoxyfen	82.5± 7.1	75.8± 6.2	85.1± 0.6	90.7± 6.9	66.5± 9.7	65.2±27.2
13	Pencycuron	68.3± 4.5	80.8± 1.7	81.2± 1.4	67.8±14.6	61.5± 9.8	70.7± 3.2
14	Fenpyroximate	82.7± 3.7	76.2± 8.9	70.6± 9.4	50.9± 4.3	69.2± 5.7	53.7± 9.8
15	Diflubenzuron*	72.7±10.9	99.5±11.6	66.9±13.3	91.5± 7.5	72.5± 3.1	80.4± 6.6
16	Hexaflumuron	70.9± 3.9	96.3± 6.2	80.9±11.7	66.9±31.1	75.6± 9.1	78.2±12.2
17	Flufenoxuron	66.0±16.2	78.9±13.0	95.8± 6.6	77.4±15.0	110.8± 4.4	76.0±20.9
18	Chlorfluazuon*	79.7±11.7	98.9± 6.2	92.0±10.2	80.6± 8.5	81.5± 9.7	85.9±15.3

*These crops were spiked with 0.5 μg/g of these pesticides and 0.1 μg/g of the others.

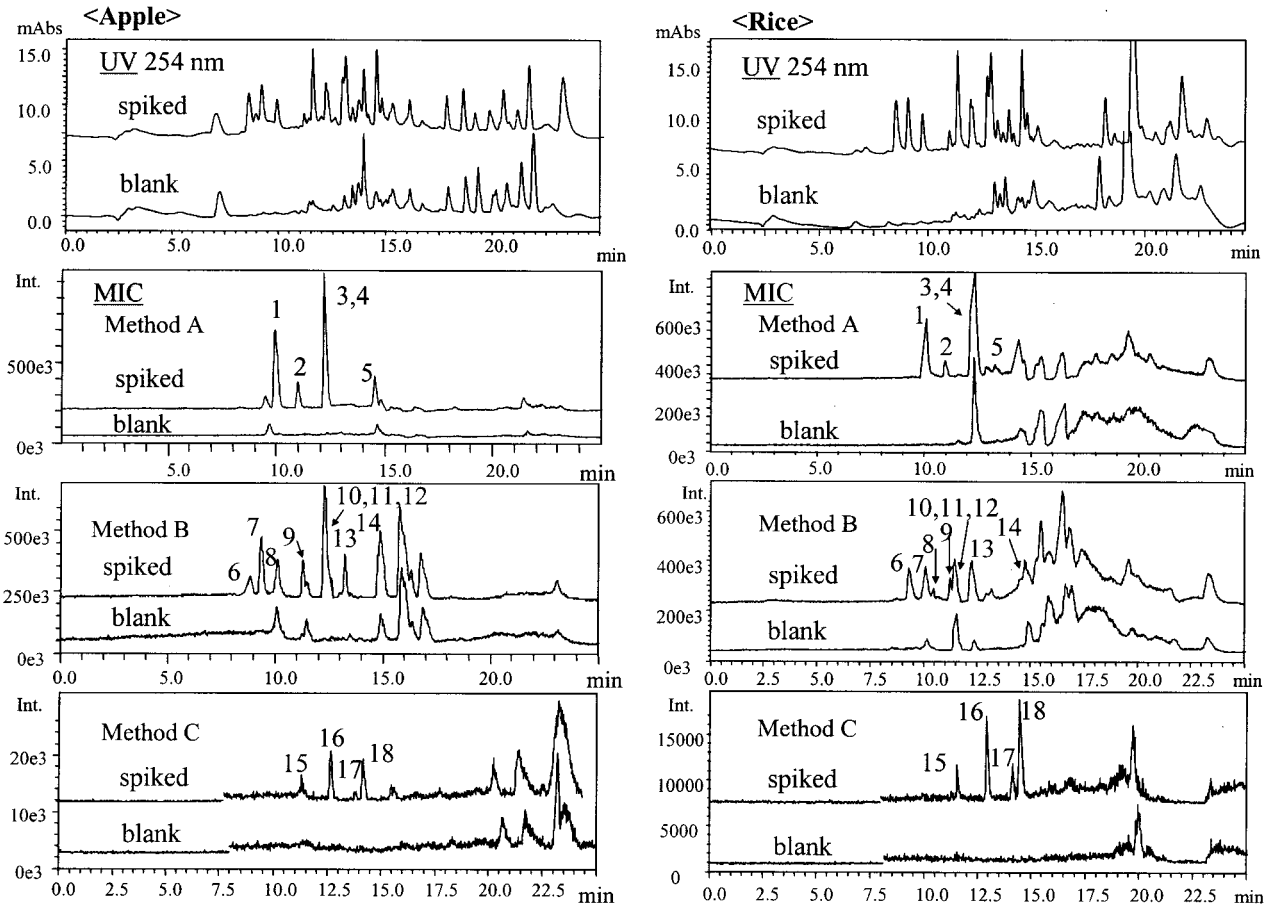

Fig. 2. HPLC (UV) and MIC of Pesticides Spiked in Apple and Rice*
*Spiked level of pesticides is the same as in Table 3.

and the method allows detecting of pesticide levels lower than the maximum residue limits.

When measuring pesticides in crops including many interfering peaks by UV detection, using the LC-MS (SIM) significantly improved the quantitative and qualitative analyses. However, sufficient maintenance of the LC-MS instrument was required in order to obtain reproducible results.

Consequently, this report could improve the drawbacks of previous report, such as long preparation time and overlapped interference peaks with target pesticides.

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